THE MICROBIAL DECOMPOSITION OF SEEDS

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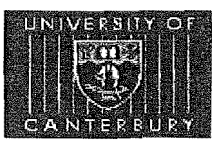

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ABSTRACT

The seed component of plant litter and its associated nutrients has been largely ignored in litter fall, decomposition and ecosystem nutrient budget studies. The exclusion of this fraction potentially underestimates the transfer of energy and nutrients within the ecosystem. This study investigated the seed substrate characteristics and the microbial decomposition of 10 species. A combination of microcosm and *in situ* experiments were used to manipulate rate-regulating factors of decomposition and measure their influence on the variables of mass loss and net nitrogen mineralisation.

Mass loss from whole seeds, decomposed under controlled conditions for 180 days, varied between 0.7% (*Sophora microphylla*) and 77.7% (*Triticum aestivum*) with the net nitrogen mineralisation varying between 0.1% (*Quercus robur*) and 67.1% (*Ulex europaeus*). The greatest amount of inter-species variation in the decomposition rate could be explained by the proportional allocation of seed mass to the seed coat fraction ($r=-0.7058$, $P=0.0001$). Alterations to the integrity of the seed coat by artificial treatments, such as scarification, heat, or grinding, and natural mechanisms, such as seed immaturity or insect damage, accelerated the rate of decay in the initial 90 days of incubation. The decomposition of seeds in combination with leaf and wood litters resulted in significant non-additive effects. The mass loss from litter mixtures exhibited both synergistic and antagonistic effects. A reduced release of nitrogen occurred in all litter mixtures. Simulated freezing and desiccation events decreased the rate of seed decomposition. The estimated mean time for 95% mass loss from whole seeds was extended from 3.6 years under constant conditions to 4.5 years and 9.0 years when exposed to cyclical wet-and-dry and freeze-and-thaw conditions respectively. The net nitrogen mineralisation was generally reduced under cyclical conditions, with the exception of the *Nothofagus* species. A mean mass loss of 43 ± 8% and nitrogen loss of 42 ±12% was measured from seeds incubated *in situ*. The effect of soil microbes on decomposition was investigated by incubating seeds under standardised temperature and moisture conditions in 3 different soils. The rate of decomposition generally declined with an increase in soil acidity.

The results of this study illustrate that the seed component of plant litter comprises a high quality substrate for microorganisms. The nutrients of seeds are generally readily mobilised and available for utilisation by other components of the ecosystem.

1 INTRODUCTION

1.1 Plant litter production

Ecosystems have evolved mechanisms to maintain and ensure the efficient internal recycling of nutrients (Odum, 1969) within its component plant, herbivore and decomposer sub-systems (Swift *et al*, 1979). Mineral nutrients are initially derived from atmospheric or regolithic sources. The quantities incurred from these external sources are negligible in comparison to the huge internal fluxes of energy and nutrients within the ecosystem. This is illustrated in the global terrestrial nitrogen cycle. The terrestrial ecosystem receives 0.20 Gt-N yr^{-1} from atmospheric deposition and nitrogen fixation. The loss of nitrogen from the system, primarily from biological denitrification and leaching, equates to 0.27 Gt-N yr^{-1}. Within the ecosystem, 332 Gt-N yr^{-1} is contained within the biota and soil (Smith, 1994). The continual recycling and redistribution of this large reservoir of nitrogen is essential for ecosystem function.

The accession of plant litter is the major pathway of nutrients and energy into the soil system. The quantity of material released is temporally and spatially variable. The production of litter is predominantly influenced by climatic conditions (Bray & Gorham, 1964). Litterfall of fine leaf and wood material in forest ecosystems can range from 15,300 kg $ha^{-1}yr^{-1}$ in tropical broad-leaved evergreen forests to 130 kg $ha^{-1}yr^{-1}$ in boreal needle-leafed evergreen forests (Vogt *et al*, 1986). Correlative studies have shown an inverse linear relationship of litter deposition mass with latitude (Bray & Gorhan, 1964; Lang & Forman, 1978; Vogt *et al*, 1986) and altitude (Heaney & Proctor, 1989).

Litter production and release will vary with season and between years (Witkamp & van der Drift, 1961; Gosz *et al*, 1972; Maggs & Pearson, 1977; Kunkel-Westphal & Kunkel, 1979; Vogt *et al*, 1986). Inter-annual variation in litter production is illustrated in *Fagus sylvatica* forest. Phillipson *et al* (1975) found the annual release of litter varied between 205-388 g $m^{-2}yr^{-1}$. The large disparity between years was attributed to the periodic production of fruit, comprising between 2% and 58% of the above-ground tree litterfall. Equatorial forests have a relatively continuous release of litter throughout the year, whereas temperate deciduous

forests senesce the majority of the leafy material in autumn coinciding with a distinct seasonal peak (Williams & Gray, 1974). A quantitative study of annual litterfall of a 200 year old *Fagus sylvatica* stand, in the Northern Hemisphere, showed that 78% of litter fell between October and December (Mason, 1977).

Plant litter consists of a diverse consort of leaf, wood, root and reproductive tissue (Mason, 1977; Swift *et al*, 1979). The quantitative and qualitative contribution of each litter component will vary between plant biomes and periodically throughout the plant life cycle. Generally leafy material is considered the major component of above-ground litter in terms of dry weight biomass (Witkamp & van der Drift, 1961; Gosz *et al*, 1972; Willimas & Gray, 1974). Lang and Forman (1978) estimated 71% of annual above-ground litterfall biomass in a mature mixed oak forest was leaf material.

Many litterfall studies have focused solely on leaf and wood material, assuming other components to be quantitatively insignificant (Miller, 1963; Meier *et al*, 1985; Vogt *et al*, 1986). The exclusion of reproductive tissue and below-ground litter has the potential to underestimate the transfer and redistribution of energy and nutrients between the vegetation and soil. The contribution of below-ground litter is probably very important in litter deposition, but it is technically difficult to study and has been neglected from many litterfall estimates. Fine root material across all climatic zones is estimated to contribute between 29-255 kg $ha^{-1}yr^{-1}$ in forests systems. Consequently, the exclusion of root litter can potentially underestimate fine litter organic matter and nutrient turnover by 20%-80% (Vogt *et al*, 1986).

The failure to include reproductive litters, such as fruits, seeds and pollens from numerous litterfall studies has been attributed to the relatively small biomass in comparison to other litter fraction; to the limited duration of the litterfall study failing to include the periodicity of phenological events; and the potential reproductive value of the fraction to the plant population. However, the comparatively high nutrient and energy content of reproductive litter and the intermittent release, generally coinciding with a period of maximum soil microbial activity during warmer months (Ovington, 1963; Carlisle *et al*, 1966; Stark, 1972), implies the reproductive component of litter is fundamental in terrestrial energy and nutrient cycling. The importance of reproductive litter in nutrient cycling is illustrated in litter measurements of *Acer saccharum* over two years. Seed production in hardwood forest

contributed greater than 1 t $ha^{-1}yr^{-1}$ dry weight biomass, thus comprising up to 34% of the total above-ground litter deposition. The mean nitrogen flux incurred from reproductive litter was estimated at 41 kg $ha^{-1}yr^{-1}$, accounting for 36% of the total above-ground litter nitrogen (Pregitzer & Burton, 1991).

The elemental composition of plant litter varies with plant life form, prevailing climate, season, age, and function of the plant tissue (Carlisle *et al*, 1966; Gosz *et al*, 1972; Berg & Staaf, 1981). Consequently, litter entering the detrital pathway displays characteristic substrate qualities, which are reflected in the decomposition rates (Swift *et al*, 1979). The decomposition rate increases from wood to leaf to reproductive tissue, regardless of climatic condition and plant life form (Swift *et al*, 1979; Meier *et al*, 1985; Prescott *et al*, 1989; Stocker *et al*, 1995), because reproductive material has a higher nitrogen content, lower lignin and phenol content than the other two litter components. Litter turnover rate, calculated as the decomposition constant (Olson, 1963), increased from 0.37, 2.55 to 9.65 in wood, leaf and reproductive material respectively (Swift *et al*, 1979). This trend was also observed in four temperate coniferous forests. Prescott *et al* (1989) observed that reproductive structures and needle material moved through the litter layer within 12 months whereas woody material remained in this layer for 2-3 years.

Leaf and wood materials undergo senescence before abscission. This nutrient-conserving mechanism mobilises carbohydrates and mineral nutrients from the tissue and redistributes them within the plant. Aerts (1996) studied the resorption of nutrients from the senescing leaves of 287 perennial species. A mean value of 50% and 52% resorption of nitrogen and phosphorus respectively was determined for all species. The percentage of leaf nitrogen pool resorbed, was significantly lower in evergreen shrub and tree species than deciduous species, whereas phosphorous resorption did not vary between growth forms. As a consequence, the senesced plant tissue has a reduced nutritional value compared with plant tissue that does not undergo senescence, such as pollens, seeds, or green material (Gosz *et al*, 1972; Kozlowski, 1973; Scott *et al*, 1992).

1.2 Plant litter microbial decomposition

The plant residue in the litter and soil represent a large pool of energy and nutrients within the ecosystem. The turnover and the mobilisation of the associated nutrients and energy are dependent on the complex process of decomposition. The simultaneous action of leaching, mechanical weathering and the comminutive and oxidative activity of the soil biota ultimately reduces the organic material to carbon dioxide, water and mineral compounds.

Microbes dominate the process of organic matter decomposition (Mason, 1977). The intrinsic properties of microorganisms facilitate the efficient turnover of organic residues. Microbes are ubiquitous in the soil environment and are capable of respiration in a broad range of habitats (Bianchi & Bianchi, 1995). Microbes have a large surface area to volume ratio. This allows intimate contact and exchange of materials between the cell and the environment. Collectively, microbes have a diverse enzymatic capacity to oxidise all natural organic substrates as a carbon source (Stolp, 1988; Heal *et al*, 1997; Richards, 1987). The exoenzymes of bacteria and fungi are either released into the soil solution or remain bound to the surface of the microbe's plasma membrane. These enzymes catalyse the hydrolysis of high molecular weight compounds such as polysaccharides, proteins and lipids into their constituent units. These are absorbed into microbial cells, providing energy and nutrients for metabolism, growth and maintenance of microbial cellular organisation.

The substrate quality and physico-chemical environment are recognised as driving variables of microbial decomposition. These factors interact in a complex manner through a series of feedback loops to influence the composition and activity of the soil microbial community (Swift *et al*, 1979). In this way, the dynamic nature of the environment and organic substrates at a microbial scale regulate the pattern and rate of decomposition and thus the release of nutrients (Swift *et al*, 1979; Berg & Staaf, 1981).

Substrate quality encompasses the physical properties and chemical characteristics of the organic substrates (Swift *et al*, 1979). Variations in these intrinsic properties alter the accessibility and availability of organic substrates to the degradative microbial enzyme and hence their rate of decomposition. The chemical characteristics of plant litter have been graded along a continuum of high to low quality substrates. Readily metabolised material,

such as sugars and water-soluble compounds, constitute high quality substrates, whereas more recalcitrant materials, such as aromatic polymers, lignins and phenols, represent low quality substrates.

The accessibility and the organic forms of elements will determine the rate of litter turnover and nutrient mobilisation. During the process of microbial catabolism, organically bound nutrients are mineralised to inorganic forms. Elements surplus to microbial requirements are released into the surrounding medium and available for immobilisation into other microbial biomass; leached to lower soil horizons; converted to volatile compounds; complexed to form more refractory compounds (Berg *et al*, 1995); or assimilated by plant roots (Russell & Russell, 1973).

Microbial mineralisation and immobilisation of nutrients are opposing processes occurring simultaneously in the soil. The balance between these processes governs the rate of nutrient release. This will be explained in the context of nitrogen mineralisation from litter substrates, as nitrogen is the key nutrient limiting primary production in most terrestrial ecosystems. Greater than 90% of nitrogen in most soil surfaces occur in an organic form unavailable for plant assimilation (Rosswall, 1976; Kelley & Stevenson, 1996). The acquisition of nitrogen is essential for cell function due to its integral role in the synthesis of protein and nucleic acids. Consequently, the plant system is highly dependent on microbial mineralisation of litter as its primary source of nitrogen. The oxidation of carbonaceous litter will promote the increment of microbial biomass. Mineralised nitrogen is accumulated into new microbial protoplasm, resulting in net nitrogen immobilisation. Conversely, the degradation of nitrogenous substrates results in net nitrogen mineralisation. The microbial population is primarily limited by energy, therefore excess nutrients will be mineralised and released into the surrounding medium resulting in the accumulation of nitrate and ammonium ions (Berg & Staaf, 1981). As the litter substrate is decomposed, a concomitant change in the proportion of carbon and nitrogen also occurs. As a consequence the predominance of mineralisation or immobilisation will be also be affected. This general model of nitrogen release is more complicated in nature due to the various organic forms of nitrogen with varying availability to microbial enzymes (Staaf & Berg, 1982).

The physical properties of the substrate will alter the rate of decomposition by affecting the resistance to herbivory, microbial degradation and physical damage. Factors such as animal comminution, expansion and contraction of soils and mechanical abrasion can act to reduce the particle size and increase the surface area to volume ratio (Swift *et al*, 1979). Maraun and Scheu (1995) found mechanical fragmentation of *Fagus sylvatica* leaf litter significantly enhanced microbial respiration in comparison to intact leaves. The surface properties, such as hard cuticle, thick epidermis and protective waxes, can retard the colonisation and infection process of microorganisms (Swift *et al*, 1979). These physical characters are effectively an index of the substrate's chemical characteristics. Numerous studies have used values of leaf 'tougness' and 'thickness' as indictors of the rate of decomposer utilisation (Witkamp & van der Drift, 1961; Maggs & Pearson, 1977; Gillon *et al*, 1994; Cornelissen & Thompson, 1997). Gallardo and Merino (1993) investigated the decomposition and substrate quality of nine Mediterranean shrub and tree species. Weight loss of leaf litter was negatively correlated with leaf 'toughness'. The variation in leaf toughness was largely associated with leaf lignin and cutin content illustrating the inter-relatedness of the physical and chemical substrate characteristics. The cutin content infers impermeability and partial resistance to microbial degradation presumably through affecting mechanical penetration of fungal hyphae.

The substrate changes physically and chemically over time as it is processed by the decomposer community and altered by the abiotic environment. These changes will have a selective effect on the composition of microorganisms capable of utilising its associated forms of energy and nutrients (Heal & Dighton, 1985). Cosmopolitan 'r-selected' microbes dominate the initial stages of decomposition. These microorganisms are competitively superior at exploiting readily degradable water-soluble materials, rich in proteins and sugars. As degradation proceeds, the proportion of structural and secondary compounds increases (Berg & Staaf, 1981; Berg *et al*, 1995). The catabolism of these more refractory cellulose, hemicellulose and lignin compounds is carried out by specialised groups of 'K-selected' microbes adapted to utilising the low energy components of the organic matter (Heal & Dighton, 1985; Bianchi & Bianchi, 1995; Heal *et al*, 1997)[1]. This is demonstrated in the

[1] Ecological theory of MacArthur and Wilson (1963) described organisms along a continuum of evolutionary strategy. At one end, 'r-selected' species display a short life span with a large reproductive effort. The other end consists of long-lived 'K-selected' species, which invest only a small proportion of resources into reproduction.

succession of biodegradative microorganisms colonising needle litter. The needles are initially colonised by bacteria and Ascomycetes that consume sugar, starch and low molecular weight extractives. The later stages are dominated by Basidiomycetes that have enzymatic capacity to degrade both polysaccharides and modify lignin matrixes (Hammel, 1997).

In nature the decomposition of the litter fractions do not occur in isolation. The plant litter consists of woody, leafy, root and reproductive material. Each litter component has a decay rate reflective of its physical and chemical properties (Swift *et al*, 1979). The decay rate of a consort of litter varies from the sum of the individual components. The different litter components will provide alternative or complementary substrates to the microbial community, consequently altering the rate and pattern of decomposition. Many inter-species studies on litter combinations have documented a 'mixture effect'. The presence of multiple litter types either enhances (Taylor *et al*, 1989b; Briones & Ineson, 1996; McTiernan *et al*, 1997; Chadwick *et al*, 1998) or retards (Finzi & Canham, 1998; Bardgett & Shine, 1999; Robinson *et al*, 1999) their respective decomposition rates. The mechanism causing these non-additive effects has been speculated to involve nitrogen, calcium, lignin, phenols and tannins (McTiernan *et al*, 1997).

Numerous studies have attempted to identify relationships between chemical attributes and the rate of substrate decomposition. Total carbon, total nitrogen, total phosphorus, carbon to nutrient ratios, lignin content, labile extractives and phenol content have been used in previous studies as indices to predict substrate decomposition rates (Swift *et al,* 1979; Berg & Staaf, 1981; Taylor *et al*, 1989a; Vogt *et al,* 1986; Hobbie, 1992), with varying degrees of success. The general relevance of such indices to the range of litter types and species is limited due to the spatial complexity and temporal variability of organic compounds within litter.

On a global scale, the rate of decomposition is strongly influenced by the climatic conditions displaying distinct latitudinal (Vogt *et al*, 1986) and altitudinal trends (Schinner, 1983; Richards, 1987). Organic matter decays more rapidly with increasing regional and seasonal temperatures (Witkamp & van der Drift, 1961). This enhanced turnover rate is strongly correlated with the energy and moisture available to soil biota, as indexed by actual

evapotranspiration (Meentemeyer, 1978). Within climatic biomes the local variations in litter turnover primarily reflects differences in the chemical and physical properties of litter substrates (Vogt *et al*, 1986).

The interaction of moisture and temperature on a local scale will determine the pH, humidity and oxygen tension of the soil environment. Microorganisms are directly affected by the external conditions due to their limited capacity to adjust their internal cell environment (Carreiro & Koske, 1992). Microbes respond to extremes in temperature and moisture either by maintaining a reduced level of activity; or by cessation of cell metabolism resulting in microbial death; or by the formation of resistant structures such as endospores, cysts, chlamydospores or sclerotia.

Temperature influences the rate of enzymatically driven oxidative reactions. Increases in temperature, within the physiological limits of mesophilic soil microorganisms (Dickinson, 1974), positively effects the decomposition rate (Witkamp, 1966; Witkamp, 1969; Anderson, 1973; Boddy, 1983; Mathes & Schriefer, 1985; Carreiro & Koske, 1992; Hobbie, 1996). The positive effect of temperature is illustrated by a decomposition study of *Agrostis festusca*. The carbon mineralisation of litter material, over a 12 week period, increased from 17% to 48% as temperature was elevated from 5°C to 30°C respectively (Floate, 1970).

The soils of Arctic and temperate regions are covered in snow or frost for a substantial part of the year. In these climates, microbial respiration continues at low temperatures. A considerable portion of the annual carbon mineralisation occurs during the winter months. This can be attributed to either the persistence of low temperatures for the majority of the year (Sommerfeld *et al*, 1993; Schimel & Clein, 1996) or the water content of the soil in the summer months being below that required for microbial respiration (Coxon & Parkinson, 1987). It is often assumed that at subzero temperatures microbial activity ceases, however respiration has been recorded at –6.0°C (Coxon & Parkinson, 1987). Microbial respiration can continue at a reduced rate in the presence of free water (Schimel & Clein, 1996). Carreiro & Koske (1992) measured the microbial decomposition of deciduous leaf litter at 0°C for 90 weeks. The leaf litter incurred mean mass losses of 13.5% and 30.7% at temperatures of 0°C

and 20°C respectively, illustrating the reduced, but continued microbial catabolism at freezing temperatures.

The environmental conditions select for distinct microbial communities with physiological adaptations to persist. Latter and Heal (1971) surveyed the activity of microbial isolates from Arctic and temperate soils. Isolates from colder sites showed a greater tolerance of low temperatures. The genera of bacteria isolated were consistent in all sites. This suggested the mesophilic bacteria were adapted to low temperatures by persisting in a state of reduced activity until environmental conditions were favourable. In contrast to this, the fungal diversity declined markedly in the colder sites. Carreiro and Koske (1992) also described composition changes in the microfungal community and a decline in the diversity of temperate microfungal communities as the temperature decreased. The mycoflora of temperate deciduous litter, at temperatures between 0°C and 10°C, consisted of relatively more Zygomycetes, and fewer Deuteromycetes species than litter communities at 20°C.

Moisture influences the growth and activity of microorganisms directly as free water is required for metabolism, growth and dispersal. Most soil fungi and bacteria activity is restricted at water potentials below –15 and –1.5 MPa respectively. *Aspergillus* and *Penicillium* species are able to maintain activity at –4 MPa (Swift *et al*, 1979). These xerophilic floras, with cosmopolitan distribution, presumably play an important role in decomposition under conditions of low moisture availability.

The control of moisture on the decomposition process is often difficult to separate from temperature and aeration effects in the natural environment. Increasing the moisture status of soils and litters has positive, negative and null effects on decomposition. For example, Boddy (1983) measured a linear increase in carbon dioxide evolution with increasing moisture in decomposing wood. However, at high moisture levels decomposition was impeded, presumably due to the restricted diffusion of oxygen and the production of anaerobic conditions. Conversely, studies investigating the decomposition process have often shown minimal effects with variations in moisture (Floate, 1970; Anderson, 1973; Mathes & Schriefer, 1985). The measure of carbon dioxide evolution over 12 weeks from decomposing *Nardus* litter at 25% and 100% moisture holding capacity varied only slightly from 17% to 20% respectively (Floate, 1970).

The soil surface is the interface between the soil and the atmosphere. As a consequence abiotic conditions at the surface closely reflect the atmospheric conditions. The components of the soil surface will be exposed to diurnal and seasonal fluctuations in temperature and moisture. The respiration of the soil litter closely parallels these changing conditions. Witkamp and Frank (1969) measured daily cycles of carbon dioxide evolution from the litter of *Pinus echinata*, *Acer rubrum* and *Quercus alba*. These peaked in the afternoon, corresponding to diurnal peaks in the temperature. These oscillating conditions decreased with increasing soil depth due to the insulating effect of the litter layers. The diurnal temperature fluctuation decreased 7.5-fold from the atmosphere to a 25 cm depth in the mineral soil of the *Pinus echinata* stand (Witkamp & Frank, 1969).

Episodic stresses, such as wet-and-dry and freeze-and-thaw cycles, can both promote and restrict the rate of decomposition by affecting the activity and composition of the microbial community and the physical properties of the litter. Stress events can reduce microbial diversity (Latter & Heal, 1971) and favour the microorganisms adapted to extreme conditions (Schimel *et al*, 1999). A single wet-and-dry cycle on birch leaf litter reduced microbial activity by 25% for two months following the disturbance event (Schimel *et al*, 1999). Fluctuations in the moisture and temperature can cause structural weakening and fractures in plant surface cells. Witkamp and Olson (1963) speculated that the periodic drying of *Quercus alba* leaves increased the brittleness and palatability to soil fauna. This favoured the fragmentation and rapid decomposition of *Q. alba* leaves.

Periodic drying and freezing of the soil has been associated with an increased carbon dioxide production following rehydration and thawing (Bottner, 1985; Kieft *et al*, 1987; Skogland *et al*, 1988; Van Gestel *et al*, 1993; Schimel & Clein, 1996). This increase has been attributed to the disruption of soil aggregates, thereby exposing interior surfaces to microbial action (Taylor & Parkinson, 1988), and the sterilisation effect on the microbial community. Low moisture and temperature conditions are lethal to many mesophilic soil microorganisms (Chen & Alexander, 1973). Surviving microbes rapidly respond to favourable changes in the environment and utilise the readily degradable substrates of dead microbial biomass. This produces a characteristic flush of carbon dioxide (Jager & Bruins, 1975; Orchard & Cook, 1983; Kieft *et al*, 1987; Skogland *et al*, 1988; Clein & Schimel, 1994). The extent of this

flush is dependent on the soil organic matter content, the substrate chemistry (Bottner, 1985) and the length and periodicity of the stress events (Schimel *et al*, 1999).

Correlates of measured site variables have little relevance to the conditions actually experienced by microbes in the soil. The influences of environmental factors are a function of the organism size. Therefore, the activity of microbes is determined by the conditions in the small, discrete and transient microhabitat it occupies. The difficulty in measuring physical variables at such a fine scale has prevented resolution of the physical factors directly affecting microbial activity. Instead correlates of decomposition and physico-chemical factors are measured at a much coarser scale and assumed to be representative of the microsite conditions.

1.3 The seed component of reproductive litter

1.3.1 Seed production and composition

Seeds are units of reproduction, dormancy and dispersal. The seed unit is released into the environment and dispersed spatially (via abiotic and biotic dispersal agents) or temporally (by the persistence in aerial and soil seed banks) (Grime & Hillier, 1992). The pattern and quantity of viable seeds released into the environment is an important factor influencing the distribution and persistence of plant populations.

Large quantities of seeds are released periodically into the soil, either directly from seeding events or indirectly via the activity of dispersal vectors or predators. The size of the seed crop depends on the plant species, age, and site factors (Koski & Tallqvist, 1978). Further, annual production of seeds can vary substantially between years as shown in Table 1.1. The large yearly variation demonstrated by *Acer saccharum* and *Fagus sylvatica* species is due to the synchronous release of vast quantities of seeds, over large geographic areas, in mast seeding events. The massive fluctuations in seed production is hypothesised as an adaptation to synchronise seedling establishment with favourable environmental conditions; to satiate seed predators (Fenner, 1985); and to provide a nutritionally favourable environment for growing seedlings (Zackrisson *et al*, 1999).

Table 1.1 Annual biomass and nitrogen contribution of reproductive litter to above-ground litter deposition.

Vegetation	n	Reproductive component	Biomass (Kg ha^{-1} yr^{-1})	Litterfall biomass (%)	N content (Kg ha^{-1} yr^{-1})	Litterfall N (%)	Reference
American Hardwood forest	1	Fruit	165	3	2.8	5	Gosz *et al*, 1972
Fagus sylvatica	8	Fruit	195-1960	1-37	ND	ND	Nielsen, 1977
Acer saccharum	2	Flower & fruit	306-1617	6-34	5.5-41.5	14-61	Pregitzer & Burton, 1991
Quercus ilex stand	4	Flower & fruit	327-781	11-24	12.6	38	Rapp, 1973
Mixed *Quercus* stand	1	Flower & fruit	68.8	11	2.1	21	Lang & Forman, 1978
Turraeanthus africanus & *Heisteria parviflora*	2	Flower & fruit	410-1320	4-14	12-16	11-14	Bernhard, 1970
Australian tropical rainforest	5	Fruit	1882	17	ND	ND	Stocker *et al*, 1995
Guatamalan tropical rainforest	2	Fruit	380-1400	8-15	ND	ND	Kunkel-Westphal & Kunkel, 1979
Amazonian tropical rain forest	1	Flower & fruit	270	3	3.3	3.8	Proctor *et al*, 1983
Amazonian tropical rain forest	3	Flower & fruit	400-700	5-8	4.1-9.4	4-9	Klinge, 1977

Note: Litterfall biomass (%) and litterfall N (%) indicates the percentage of total litter biomass and nitrogen accounted for by reproductive material respectively. n = number of years litterfall was measured. ND = not determined.

Seeds consist of a nutrient- and energy-rich endosperm, and a protective seed coat. Reserve material is primarily stored in the seed endosperm or cotyledons. Seed reserves consist of carbohydrates (sugars, oligo- and polysaccharides), proteins, lipids, organic phosphates and various inorganic compounds, that together function to supply the developing embryo with carbon and nutrients. Starch is the predominant reserve material in many cereals such as wheat, rice and maize. Smaller seeds generally contain lipid reserves, such as glycerides or fatty acids. This is prescribed to the lipid compounds high caloric value per unit of mass. Storage proteins are located in protein bodies or microbodies. Proteins are classified according to solubility into albumins, globulins, prolamins and glutelins. The principle proteins in most seeds are globulins. The exception is in grass seeds, such as cereals. Proteins in cereals account for about 8-15% of the grain dry weight, of which 40-60% are prolamines and 20-40% are glutelin (Bryant, 1985; Copeland & McDonald, 1995).

The outer layers of seeds function as a protective barrier separating the embryo from the external environment (Fenner, 1985). The seed coat is a multi-layered membrane, generally consisting of a fatty or waxy outer cuticle over lying layers of thickened cells. These layers confer properties of selective permeability thereby regulating the physical and chemical environment experienced by the embryo (Bewley & Black, 1978). The outer layers may restrict microbial colonisation. The seed coat presents a physical barrier to hyphae penetration and the movement of low molecular weight compounds from the internal seed environment, thereby presenting a substrate of low nutritional value (Halloin, 1983).

The seed coat and endosperm vary with respect to function and thus their substrate quality. The protective function of the seed coat is reflected in the increased lignin and structural carbohydrate content and the reduced nitrogen and water-soluble content (Lee *et al*, 1991; Larson, 1968; Grubb & Coomes, 1997). The endosperm material, due to its high carbohydrate, lipid and protein content, is effectively a concentrated unit of energy and nitrogen. These chemical characteristics of the seed coat and endosperm are indicative of low- and high-quality microbial substrates respectively. Consequently, the collective seed substrate will presumably require a consort of K- and r-selected microbes to co-metabolise the associated energy and nutrients (refer to section 1.2). The variation found in seed size, chemical composition and fractionation of the seed components, would presumably result in divergent substrate qualities.

The effectiveness of the seed coat as a barrier to the external environment is jeopardised by the presence of natural openings, congenital defects and mechanical or chemical injuries to the seed coat (Baker, 1972; Halloin, 1983). Acid hydrolysis in the soil or animal digestive tract (Copeland & McDonald, 1995), fluctuating temperature and moisture conditions (Fenner, 1985; Zammit & Zedler, 1994; Jansen & Ison, 1995), mechanical abrasion (Williams, 1981) and microbial degradation of the outer seed layers (Gogue & Emino, 1979; van Leeuwen, 1981) have been implicated as natural scarifying processes. Changes in the integrity of the seed coat can cause damage to the seed embryo, increase the movement of water and gases stimulatory to seed germination, and provide entrance sites for degradative microbes and their associated enzymes. Mechanical damage to peanut seeds reduced seedling

emergence by 66% (Bell, 1974). This reduction was attributed to the exposure of seed and embryonic tissue to soil fungi.

Natural openings in the seed coat assist in the movement of materials between the external environment and the seed. These sites also provide entrance points for pathogenic and saprophytic microorganisms (Baker, 1972). Cotton seeds possess thick seed coats, but at the point of attachment, there is a small plug that extends into the internal tissue providing an entrance site for fungi (Christensen, 1973). Kremer (1987) observed 90 % of the internal bacteria isolated from *Abutilon theophrasti* seeds were preferentially associated with the natural seed openings and its surrounding tissue.

1.3.2 Allocation of nutrients and energy to seed production

The production of seeds represents a considerable demand on plant energy and nutrient resources that can lead to a reduction in vegetative growth or survival rate (Bazzaz *et al*, 1979). The seeds act as a sink and assimilate storage material synthesised elsewhere in the plant. The plant provisions seeds with resources to support seedling growth, with little guarantee that the seed will successfully establish as an autotrophic plant. Falinska (1971) estimated 30-40 kg N $ha^{-1}yr^{-1}$ was needed for the production of reproductive tissue in a *Querco-Carpinetum* ecosystem. The source of nitrogen is either from the relocation of stored reserves or the uptake of organic nitrogen in the soil (Pregitzer & Burton, 1991) which ultimately deprives vegetative growth now or in the future.

The allocation strategy employed by the plant is species- and site-specific. The total reproductive effort, timing and periodicity are key factors in determining the individual's contribution to future generations (Fenner, 1985; Bazzaz *et al*, 1987). The plant must allocate finite nutrient and energy resources between reproductive and vegetative growth to maximise plant fitness (Bazzaz & Ackerly, 1992). This trade-off is illustrated in plant physiological studies. Dick *et al* (1990) demonstrated that male cones of *Pinus contorta* were acting as photosynthetic sinks. Branches bearing male cones had 33% fewer needles than the vegetative branches. Lee and Fenner (1989) found three of the four tested *Chionochloa* species showed a reduction of tiller weight in flowering tussocks. Litterfall studies of species that undergo mast seeding events often observe negative correlations between foliar and

reproductive litter inputs implying a vegetative and reproductive trade-off (Nielsen, 1977; Pregitzer & Burton, 1991).

1.3.3 Seed persistence in the soil system

The accumulation of viable seeds in the soil and the associated litter collectively comprises the soil seed bank (Simpson *et al*, 1989). The quantity and composition of the seed bank is determined by the replenishment of seeds from the above-ground vegetation via seed rain and dispersal, the dormant seeds present in the soil, and the losses incurred from germination and seed death.

Most temperate and tropical habitats have some form of persistent seed bank (Murdoch & Ellis, 1992). The total amount of transient and persistent seeds within the soil varies substantially between ecosystems. For example, the seed banks of arable land vegetated with small grain cereal crops ranged from 4,742-73,350 seed m^{-2} (Cavers & Benoit, 1989). Comparitively, Mladenoff (1990) measured only 4 seed m^{-2} in a temperate hardwood forest dominated by *Acer saccharum*, *Tsuga candensis*, and *Betula alleghanensis*. Generally the number of seeds present in the soil declines with latitude, altitude, and successional age of the ecosystem (Pickett & McDonnell, 1989; Grime & Hillier, 1992; Baskin & Baskin, 1998).

Seeds are able to retain viability in the soil for long periods of time by going into a state of quiescence or dormancy. The longevity of seeds in the soil was demonstrated by Beal's experiments of 1879. A selection of seeds mixed with soil and moisture were able to retain their ability to germinate after 100 years burial (Priestley, 1986). The most persistent seeds are generally characterised by a small compact shape (<3mg) and smooth seed coat (Chambers, 1993; Baskin & Baskin, 1998). The shape and size of the seed is speculated to both promote seed burial and reduce predative pressures from granivores (Thompson *et al*, 1993).

The persistence of seeds is related to their specific germination and dormancy requirements, and resistance to microorganisms (Thompson *et al*, 1993). To achieve this, the plant provisions seeds with protective chemicals and physical barriers. Thus the presence of an impermeable seed coat ('hardseededness') can promote longevity by preventing the diffusion

of water and gases (stimulatory for germination), restricting embryo growth, retarding hyphal penetration, and reducing the availability of nutrients to microbes external to the seed (Harman *et al*, 1978; Halloin, 1983; Jansen & Ison, 1995).

Seeds may possess anti-microbial compounds. The seed exudates of *Abutilon theophrasti* were assayed for its inhibitory effect on 241 microbial isolates. The exudates inhibited the growth of 58% and 100% of the bacteria and fungi species respectively (Kremer, 1986b). The dark seed coat of *Pisum arvense* seeds possess anthocyanins that inhibit the penetration of *Pythium* hyphae (Halloin, 1983). However, damage to the palisade layer provides entrance points for pathogenic microorganisms. This increases the seeds susceptibility to the foot rot pathogen (Halloin, 1983). The inhibitory action of plant extracts to microorganisms is often difficult to prove in the natural environment. Identification of the causal factors often fails to separate other suppressive interactions, and conclusively show inhibition at concentrations present in the field (Wardle *et al*, 1998).

The number of seeds that persist in the soil and emerge successfully demonstrates the effectiveness of these physical and chemical characteristics against microbiota. The seeds in the soil are exposed to a battery of microbial enzymes with diverse degredative capacity. Despite these sub-optimal conditions, seedling emergence continues, subject to microsite availability. This aspect of the life history strategy of the plant is fundamental for the species persistence.

The retention of seed viability over time has important implications for the management of disturbed and natural ecosystems. An appreciation of the factors promoting seed longevity in the soil is important for agronomists in controlling weed species (Priestley, 1986; Thompson, 1992) and ecologists in understanding the regeneration and maintenance processes of plant populations (Fenner, 1985; Baker, 1989; Wilson, 1990; Gilfedder & Kirkpatrick, 1993; Moles & Drake, 1999).

1.3.4 Seed losses incurred throughout the development, dispersal, persistence, germination and establishment stages

Seed that fail to germinate and successfully establish as a seedling represents a loss of reserves to the parent plant. Seed resources can be lost throughout the seed development, dispersal, germination, and establishment processes.

Premature abscission can occur through pollination failure, resource deficiency, extreme temperatures, drought, fungal attack (Christensen, 1973), and invertebrate and vertebrate damage (Kozlowski, 1973; Cavers, 1983; Fenner, 1985). Abscission of immature fruits is a significant direct loss of nutrients to the plant. It is estimated that 90% of potential acorns of *Quercus alba* are aborted and released during pollination, fertilisation and ovule development (Kozlowski, 1973).

Throughout the life of the seed, degradation processes occur and accumulate eventually resulting in physiological death (Abdul-Baki & Anderson, 1972). As seeds age, biochemical changes occur within the seed (Priestley, 1986). These include changes in seed ultrastructure (Anderson & Baker, 1983), lesions and fragmentation of genetic material, altered activity of enzymes (St. Angelo & Ory, 1983), reduction in respiration rate, and changes in membrane integrity (Abdul-Baki & Anderson, 1972; Copeland & McDonald, 1995). Some species, in an imbibed state, demonstrate a capacity to synthesise membranes and organelles and maintain macromolecules to counteract these deteriorating processes (Priestley, 1986). However, this repair capacity is exceeded by the continued accumulation of biochemical degradation products (Baskin & Baskin, 1998). The susceptibility of seeds to deteriorating processes varies with the environmental conditions (Abdul-Baki & Anderson, 1972). These environmental factors are manipulated in agriculture to extend the longevity of crop seeds. Seeds are generally stored in conditions of low moisture, cool temperatures and low oxygen tension to retard seed ageing and reduce the associated fungal colonisation (Christensen, 1973; Harman, 1983).

The net effect of deteriorating processes is expressed at the morphological and physiological level. Seeds may show discoloration, changes in seed coat structure, delayed emergence, decreased tolerance of stressful conditions during germination, reduced seedling vigour and

ultimately embryo death (Abdul-Baki & Anderson, 1972; Copeland & McDonald, 1995). Individual seeds vary in their chemistry and susceptibility to deteriorating processes, resulting in the non-uniform deterioration of seed populations (Copeland & McDonald, 1995).

The progressive degradation of seeds is generally concurrent with increased microbial colonisation. As seeds degrade, the increased loss of membrane integrity is associated with an enhanced release of seed exudates. These leachate stimulate microbial growth through the provision of readily available energy and nutrients (Harman, 1983). This may facilitate the decomposition of the more refractory seed coat compounds through a priming effect. The release of volatile compounds from degrading seeds can also stimulate microbiota. The deteriorated seeds of *Pisum sativum* produced increased quantities of volatile carbonyl compounds in comparison to fresh seeds. The production of these volatiles stimulated microbial growth and spore germination of pathogenic fungi in sand substrates (Harman *et al*, 1978).

Consumers of plants, at any stage of the plant's life cycle, have the potential to influence the release and survival of seeds. The consumer can affect plants directly by the removal of reproductive organs, or indirectly by modifying the net primary production and therefore altering the plant's ability to invest in reproduction. Quantifying the influence of consumers on plant populations is difficult. The impact is dependent on the timing of seed loss, the environmental conditions, compensatory mechanisms and the frequency of favourable microsites for seedling establishment (Louda, 1989).

Animal interactions with plants can be beneficial or detrimental to seeds. Numerous species have demonstrated an enhanced germination after passage through the gut of an animal disperser (Krefting & Roe, 1949). The ingestion of seeds results in scarification of the seed coat presumably via mechanical abrasion from gizzards, acid hydrolysis within the animals' stomach or enzymatic degradation by the animals' associated microbiota. The beneficial relationship between plant and animal is illustrated in the hypothesised obligate mutualism between *Calvaria major* and the extinct dodo bird, *Ralphus cucullatus*. Circumstantial evidence from fossil records and the reduced *C. major* recruitment post-*R. cucullatus* extinction, suggests abrasion of the thick endocarp of *C. major* was dependent on ingestion

by *R. cucullatus*. In the absence of *R. cucullatus*, no natural germination has been observed (Temple, 1977).

Alternatively, animals can increase seed mortality through damage to the seed embryo and comminution. Consumption by specialist invertebrates can reduce seed crops by 90%-100% (Louda, 1989). Microorganisms may act in conjunction with insects to deteriorate seeds (Mills, 1983). The insect facilitates microbial colonisation by acting as a vector, dispersing and inoculating the plant tissue with microorganisms. The feeding activity of insects will make seed substrates available to microorganisms either directly, by rupturing the seed coat and making seeds vulnerable to microbial invasion (Janzen, 1977), or indirectly via the insects' excretion products. The seasonal infestation of a seed-feeding insect, *Niesthrea louisianica*, was shown to significantly reduce seed viability and increase the incidence of fungal infection from 8% to 98% in *Abutilon theophrasti* (Kremer & Spencer, 1989). The insect feeding activity breached the physical barrier of the hard seed coat thus facilitating microbial colonisation. Scanning electron micrographs showed fungal hyphae and bacteria cells were intimately associated with stylet punctures in the seed. The manipulation of such insect-microbe interactions has been advanced as a strategy in the biocontrol of weed species.

After release from the parent plant, seed reserves may be lost from the soil through scarifying mechanisms forcing the process of seed germination or seed death (Simpson *et al*, 1989; Lonsdale, 1993; Baskin & Baskin, 1998). The survivorship is species-specific and declines in a negative exponential manner over time (Leishman *et al*, 2000). Extensive losses of seeds have often been observed in studies of seed dynamics in the soil. Crist and Friese (1993) showed a rapid decline in shrub-steppe viable seed bank over 10 months. The viability of the confined seeds within the soil declined from 84% to 4%. The mechanism of seed loss and factors contributing to seed survival remain relatively obscure. Germination failure (Nielsen, 1977; Zackrisson *et al*, 1999), extreme physical conditions such as flooding and fire (Baskin & Baskin, 1998), physiological death (Abdul-Baki & Anderson, 1972), animal predation (Cavers, 1983; Louda, 1989), pathogen attack (Baker, 1972; Baker, 1989) and microbial decomposition (van Leeuwen, 1981; Crist & Friese, 1993) have been implicated as mechanisms of seed loss. Through the action of these depletion processes, up to 90% of seeds are rendered non-viable and eventually enter the detrital pathway (Leishman *et al*, 2000).

Like other litter components, it is difficult to identify a definitive time when the plant tissue is subjected to microbial decomposition. Decomposition processes by seed-borne microflora can occur while the seeds are still attached to the parent plant (Baker, 1972). Saprophytic fungi release hydrolysing enzymes that deplete the endosperm reserves, partially or entirely decaying the seed (Cherry, 1983). This is further confounded by our inability to precisely diagnose when a seed ceases to be of ecological value to the regeneration and persistence of the plant community and enters the detrital pathway. Seeds are capable of germination even if the embryo is partially damaged or the endosperm depleted.

1.3.5 Microbial decomposition of seeds

Very few studies have resolved or quantified the causative factors of seed loss in the soil. Circumstantial evidence of the microbial decomposition of seeds is provided by 1) the ubiquitous presence of microbes on seeds; 2) the degradative capability of microorganisms to utilise seed substrates; 3) the speculated and observed role of microorganisms in seed mortality within the soil; and 4) the continual turnover of seeds in the natural environment. These findings implicate microorganisms as a significant factor in determining the fate of seed reserves in the soil.

All seeds are contaminated by microorganisms of seed or soil origin (McGee, 1983). Taxonomic studies have shown both generalist (Crist & Friese, 1993) and specialist microbial associations on the seed substrates (Kirkpatrick & Bazzaz, 1979; Kremer, 1987). Variations in the composition and diversity of microbial populations between species imply structural, physiological and chemical selectivity of the seed substrates. The presence of microorganisms can be detrimental, resulting in seed decay and causing a loss of seed viability. Alternatively microbes can serve a protective function. Internal seed bacteria, in the subpallisade layer, can be antagonistic to the soil fungi, thus inhibiting fungal invasion (Kremer, 1986a).

Seeds represent a concentrated unit of energy and nutrients to microbiota. Pathogenic and saprophytic microbes are capable of invading and exploiting high quality substrates associated with seeds. Evidence of microbial enzymatic capacity to degrade seed substrates is provided by research investigating the impact and control of storage fungi in commercial

seeds. Xerophytic microbes, predominantly *Aspergillus* and *Penicillium* species, are able to grow on seeds in the absence of free water at a relative humidity between 70-90% (Harman, 1983; Copeland & McDonald, 1995). The role of fungi in seed degradation has been demonstrated in exclusion experiments. Pea seeds stored at 85% relative humidity and 30°C for 6 months retained 97% viability in the absence of storage fungi. Seeds inoculated with fungi lost all viability after 6 months storage (Christensen, 1973). The detrimental effect of storage fungi may be from the invasion of the embryo, production of mycotoxins or the depletion of seed reserves (Copeland & McDonald, 1995).

Soil microbes are capable of degrading the seed testa. The germination of *Albizia julibrissin* was elevated from 11% to 30% by exposing seeds to soil microorgansims (Gogue & Emino, 1979). Electron micrographs implicated fungal hyphae in the degradation of surface cells, thereby promoting the imbibition of *A. julibrissin* seeds. *Cirsium vulgare* seeds show substantially higher germination rates after inoculation with seed- and soil-borne microorganisms in comparison to sterilised seeds (van Leeuwen, 1981). The scarifying role of microbes is not universal to all species and can vary through out the year. Crist and Friese (1993) proposed fungal degradation of the seed coat as the key factor contributing to seed bank losses in the colder months of the year, whereas fluctuating temperatures were the dominant factor promoting germination in the warmer months.

Numerous seed bank studies have speculated on the role of microorganisms in seed depletion (Weaver & Cavers, 1979; Kalisz, 1991; Crist & Friese, 1993). The role of fungal pathogens in the natural environment has been investigated by the incubation of fungicide-treated seeds within the soil. Fungal exclusion lead to highly variable increases in the seed survivorship. *Trifolium balansae* and *T. resupinatum* showed only a small increase of 0.1% and 0.2% respectively in seed survivorship (Jansen & Ison, 1995). The treatment of *Mimosa pigra* with fungicide decreased seed death by 10-16% (Lonsdale, 1993). The largest effect was observed for *Miconia argentea* and *Cecropia insignis* with a reduction in seed loss by 47% and 39% respectively (Dalling *et al*, 1998). These studies indicate that the role of fungi in enhancing losses of seeds from the soil is species-specific (Leishman *et al*, 2000).

A limited number of studies have provided circumstantial evidence for the decomposition of seeds in the soil environment. Germination trials have commented on the contamination of

seeds during incubation (Webb, 1993) and the role of soil microbes reducing viability (Burrows, 1991). Burrows (1993) observed a reduction in germination rate and viability when seeds were germinated in soil substrates. Alvarez-Buylla and Martínez-Ramos (1990) observed the soil environment reduced the viability of *Cecropia obtusifolia* seeds from 85% to 5%. Seed predators and pathogens were speculated as the main factors contributing to the low seed survivorship and rapid seed turnover. The nutritional effects of dead *Picea abies* seeds on Scots pine seedlings provided further evidence of seed decomposition. The pine seedlings showed an increased growth rate and nutrient content in sites fertilised with dead *P. abies* seeds, implying the nutrients of dead *P. abies* seeds are readily mineralised and available for plant assimilation (Zackrisson *et al*, 1999).

Plants have adopted various strategies to avoid invertebrate and vertebrate predation of seeds. Seeds can display chemical and physical defence characteristics to deter predators (Lee & Fenner, 1989). The plant can vary seed output between years to satiate predators. Considering microorgansims small size, ubiquitous distribution and diverse enzymatic capabilities, these strategies would be expected to have limited effectiveness against the 'predatory' behaviour of microorganisms.

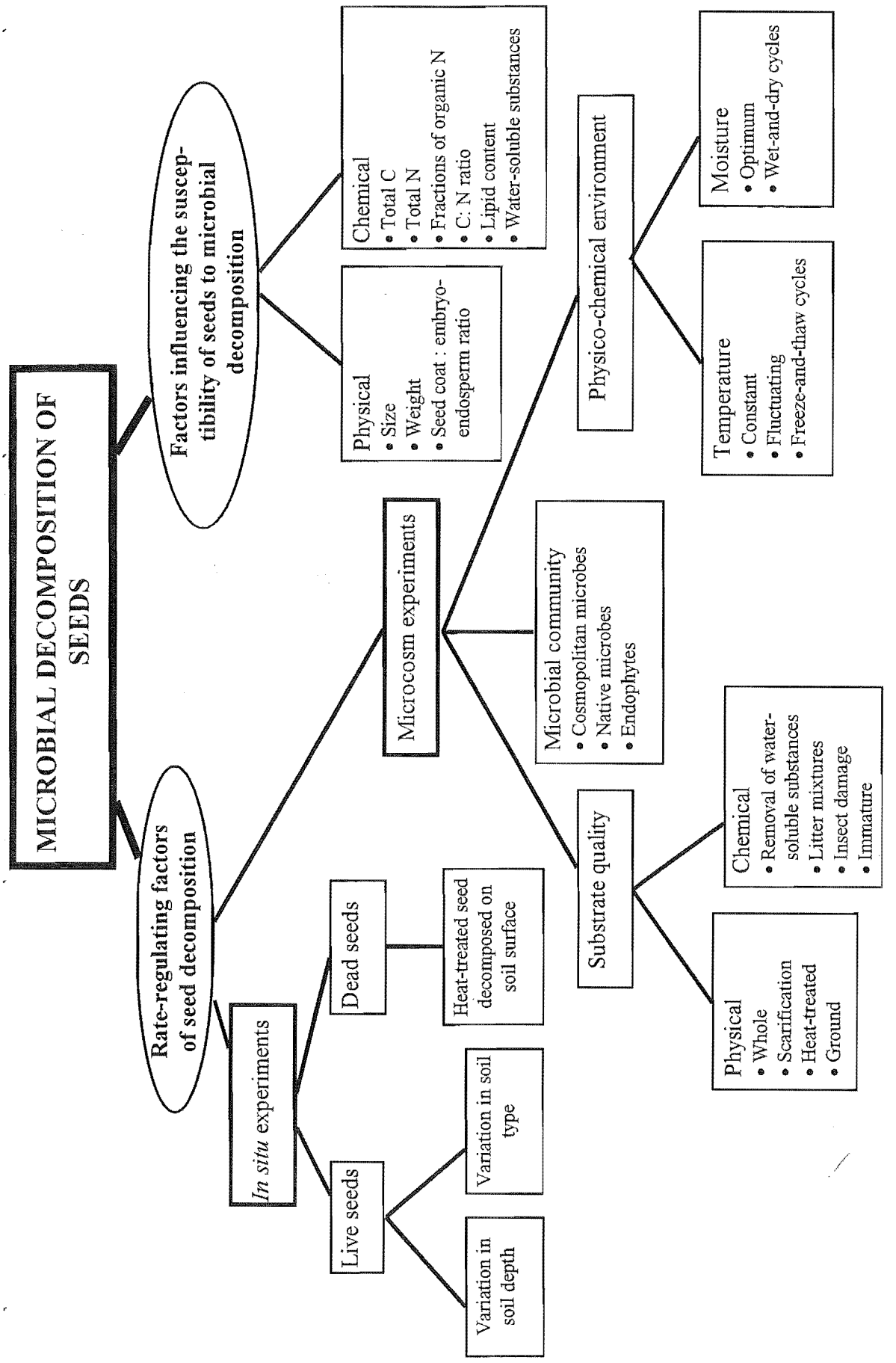
To my knowledge only one paper has directly investigated the decomposition and the concomitant release of nutrients from seeds. Greenfield (1998) provided biochemical evidence for the rapid decomposition and mineralisation of organic nitrogen in seeds. The mass loss of the surveyed seeds ranged from 8% in *Betula pendula* to 62% in *Lolium perenne* within 30 days. Soil microorganisms mineralised between 0% in *B. pendula* and 65% in *Malus pumila* of the initial seed organic nitrogen in 30 days. This study demonstrates some seed nutrients are in a form that can be rapidly mineralised and released during seed decomposition.

1.3.6 Importance of this research

Large quantities of nutrient-rich and energy-rich seeds are periodically released into the soil environment. Only a fraction of these seeds will germinate and successfully establish as seedlings. The quantity and fate of the residual seeds is largely unknown. One possible mechanism of seed depletion, that is often speculated about but has received minimal attention in the literature, is the microbial decomposition of seeds in the soil. Circumstantial evidence for microbial degradation is provided from the pathogenic and saprophytic activity of storage fungi, the scarifying role of soil microbes, the restricted germination of seeds in soil environment and the huge seed losses incurred from seed banks. It is likely that seeds are decomposed in the soil environment but there is limited direct experimental evidence. Microbial degradation of seeds is likely to play an important part in the loss of seed viability, and the subsequent cycling of plant nutrients and energy in the ecosystem.

This thesis aims to provide biochemical evidence for the microbial decomposition of seeds in the soil environment. Physical and chemical attributes of seeds will be assessed as an indicator of seed susceptibility to microbial degradation. A combination of *in situ* and microcosm experiments will be used to investigate the extent and rate of seed decomposition. Parameters of weight loss and mineral nitrogen will be measured over time as indicators of microbial metabolism and net nitrogen mineralisation, respectively. The rate-regulating factors such as temperature, moisture, substrate quality and the decomposing microbial community will be manipulated to investigate their effect on the rate and pattern of seed decomposition. This is summarised in Figure 1.1. Finally, estimates of nutrient release will be extrapolated to ecosystem level to approximate the potential contribution of seed litter to soil carbon and nutrient pools.

Figure 1.1 Summary of experiments investigating the microbial decomposition of seeds



2 MATERIALS AND METHODS

2.1 LITTER COLLECTION

2.1.1 Introduction

The species surveyed included a selection of native forest species, introduced species, and economically important agricultural and forestry species. This study focused on sub-samples of seed population to provide information on their microbial susceptibility.

2.1.2 Methodology

Seed samples were collected from several sites around the Canterbury region. Collections were carried out between late-February and early-May 2000, corresponding to the natural period of seed release for each species. An additional collection of *Ulex europaeus* occured in November 2000. Lincoln University kindly provided *Triticum aestivum* and *Hordeum vulgare* seeds. Additional samples of *Pinus contorta* and *P. radiata* were purchased commercially from Proseed Ltd at Amberly, North Canterbury. Insect damaged seeds of *Sophora microphylla* and *Ulex europaeus* were removed directly from the seedpods.

The seeds were either harvested directly from the parent plant or collected from the surface of the litter layer beneath the seeding trees. The sampling method used varied between species due to the life history and dispersal strategies employed by the respective species (Table 2.1).

The seed samples were air-dried to a constant weight for at least 7 days and stored in airtight containers or sealed polythene bags at room temperature (approximately 20 ± 2°C) under low light conditions. The *Quercus robur* seeds were recalcitrant, with a high moisture content. This species was kept in an open container to promote drying and minimise microbial activity. These conditions potentially reduced the viability of recalcitrant species (Baskin & Baskin, 1998). Handling of the seeds was kept to a minimum to reduce mechanical injury that may promote microbial invasion. All seeds were tested within 10 months of collection, as seed viability generally decreases exponentially with time (Priestley, 1986). Unless otherwise stated, mature undamaged seeds were used in all experiments.

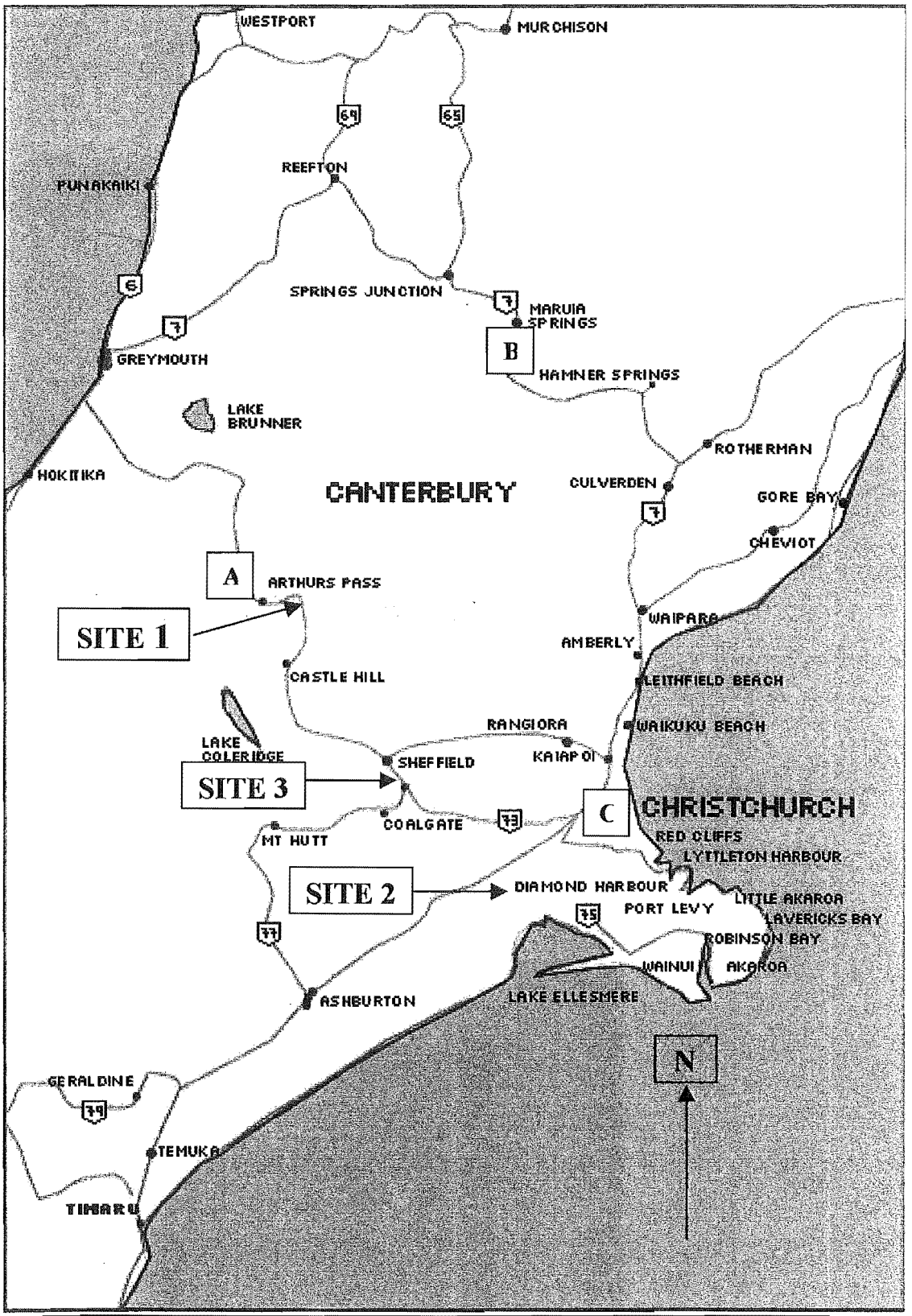
Table 2.1 Species seed characteristics, dispersal strategies, sites and methods of collection.

Species (scientific name)	Species (common name)	Dispersal strategies	Collection site	Collection method
Triticum aestivum	Wheat	Modified	Lincoln University	Supplied
Hordeum vulgare	Barley	Modified	Lincoln University	Supplied
Pinus radiata	Monterey pine	Cone, wind dispersed	Canterbury farm / Proseed Ltd, Amberly	Tree / supplied
Pinus contorta	Lodgepole pine	Cone, wind dispersed	Arthurs Pass (Craigeburn) / Proseed Ltd Amberly	Tree / supplied
Nothofagus fusca *	Red beech	Gravity	Lewis Pass (Maruia forest)	Litter
Nothofagus solandri *	Mountain beech	Gravity	Arthur's Pass (Craigeburn)	Litter
Cytisus scoparius	Scotch broom	Explosive pods, seed bank, fire	Christchurch (Port Hills)	Tree
Ulex europaeus	Gorse	Explosive pods, seed bank, fire	Christchurch (Russley)	Tree
Quercus robur	Red oak	Gravity, animal	Christchurch (University grounds)	Litter
Sophora microphylla *	Kowhai	Wind, water, soil seed bank, canopy seed bank, fire	Christchurch (Deans Bush, private garden)	Litter

Note
- * = native species.
- Tree = seeds removed directly from the tree prior to natural dispersal, Litter = seeds collected from the litter layer underneath parent tree, Supplied = seeds provided by a commercial supplier.

Leaf and woody litter (twig < 3mm in diameter) of *N. solandri*, *N. fusca*, *P. radiata* and *U. europaeus* were collected in June 2000. The litters of *N. solandri*, *N. fusca* and *P. radiata* were collected from the soil surface at Arthur's Pass (Craigeburn), Lewis Pass (Maruia), and Christchurch (University of Canterbury) sites respectively (refer to Figure 2.1). The litters were removed from the litter layer surface to obtain material that was most recently senesced and therefore subjected to minimal decomposition processes. A previously harvested branch of *U. europaeus* was collected from a Christchurch farmland. The litter factions were air-dried to a constant weight for a minimum of 7 days and stored in sealed polythene bags at room temperature (approximately 20 ± 2°C). The leaf and twig litters were used in substrate quality (Section 2.3) and 'litter mixture' (Section 2.4.4.5) experiments.

Figure 2.1 Map of Canterbury showing collection sites for litter and soil samples



Key
SITE 1: Hawdon soil. Site vegetated by mature *N. solandri.*
SITE 2: Lincoln soil. Arable land vegetated by *C. scoparius*
SITE 3: Darfield soil. Site vegetated by mature *P. radiata*
A: Craigeburn: *P. contorta, N. solandri*
B: Lewis Pass: *N. fusca*
C: Christchurch: *C. scoparius, U. europaeus, Q. robur, S. microphylla*

2.2 GERMINATION TEST

2.2.1 Introduction

Viability denotes the ability of a seed to germinate and produce a 'normal' seedling, under favourable conditions (Priestley, 1986). Viability can be measured either through biochemical tests that qualify the presence of enzymes indicative of metabolically active tissue or germination trials (Copeland & McDonald, 1995).

To determine the potential of seeds to germinate in the field, germination tests were conducted under optimal conditions either standardised by the Association of Official Seed Testing (1996) rules for testing seeds or recommended by published germination studies. This technique has the potential to overestimate germinability, as favourable conditions rarely exist in the field.

2.2.2 Germination trials

Germination tests were carried out on each species between August 2000 and January 2001. A minimum of 300 seeds for each species was used in the germination trials. The samples were divided into sub-samples ranging between 25 and 50 seeds. Unsterilised seeds were used in all germination trials as seed-borne microflora may play a significant role in seed germination (Kirkpatrick & Bazzaz, 1979; Kremer, 1986A). Table 2.2 describes the conditions and treatments carried out on the respective species to determine germination potential. Whatman No.2 filter paper was used in germination trials where the use of paper is indicated.

The seeds were checked weekly to observe germination, ensure adequate moisture and observe pathogenicity. The seeds were considered germinated when the radical emerged from the seed coat (>2mm), at which point they were removed from the germination trial. The germination substrates were renewed frequently due to excessive fungal growth. Fungal infected seeds were removed and germinated separately. After the test period was completed, seeds that failed to germinate were incubated for an additional 60 days under the recommended conditions. The remaining ungerminated seeds were inspected for microbial growth, obvious physical damage and seed hardness.

Table 2.2 Germination Test Conditions.

Species	Substrata	Temp (°C)	Time period of test (days)	Additional treatments	Reference
Triticum aestivum	TP	20	7	Preheat 30 °C for 5 days	AOST
Hordeum vulgare	BP	20	8	Preheat 30 °C for 5 days	AOST
Pinus radiata	TP	20	25	Prechill 21 days (5 °C)	AOST
Pinus contorta	TP	20	21	Paired test. Prechill 21 days (5 °C)	AOST
Nothofagus fusca	TP	15-20	90	Stratify for 60-70 days	Beggs, 1999
Nothofagus solandri	TP	15-20	90	Stratify for 60-70 days	Beggs, 1999
Cytisus scoparius	TP	20-30	35	Pierce seed testa at cotyledon end and soak DDW for 3 hours.	AOST
Ulex europaeus	TP	20-30	35	Pierce seed testa	Zabkiewicz, 1976
Quercus robur	TS	20-30	14	Cut 1/3 off scar end and remove pericarp.	AOST
Sophora microphylla	TP	10-20	90	Scarify seed coat on both lateral faces	Webb, 1993

Note

- TP = top filter paper, BP = between filter paper, DDW = double distilled water, AOST = Association of Official Seed Testing.

The viability of a species was calculated as the percentage of the initial seeds in the trial that germinated successfully, regardless of the presence or absence of microbial growth (Equation 2.1).

Equation 2.1

$$\% SV = (G / T) \times 100$$

Note:
SV = seed viability.
G = number of germinated seeds over entire test period.
T = total number of seeds included in the germination trial.

2.3 CHARACTERISATION OF SUBSTRATE QUALITY

2.3.1 Introduction

The chemical and physical properties of litter substrates are indicative of the litters value and accessibility to microbial catabolic activity (Swift *et al*, 1979). The moisture, ash, pH, organic matter, nitrogen (Kjeldahl), oxidisable carbon, lipid content and water labile extractive mass and chemistry were determined for all seed substrates. The seed mass, length, width and water retention was determined to qualify the physical attributes of each species. The seeds were fractioned into seed coat and embryo-endosperm components to investigate the proportional allocation of seed mass and nitrogen between the seed fractions.

2.3.2 Moisture, ash, organic matter and pH

Moisture, ash, organic matter and pH measurements were done in triplicate to reduce experimental error. Weighed air dry (AD) litter samples (0.5-1.0 g) were placed in pre-weighed crucibles. The crucibles were placed in an oven, set at 105°C for 15 hours, cooled in a desiccator and weighed on an analytical balance to give an oven dry (OD) moisture free mass (Chapman & Pratt, 1961). The moisture content was calculated as the percentage difference between the sample's air dry mass and oven dry mass (Equation 2.2).

Equation 2.2

$$\% \text{ moisture} = [(\text{sample AD} - \text{sample OD}) / \text{sample AD}] \times 100$$

Note:
AD = air dry.
OD = oven dry.

The litter samples were subsequently placed in a muffle furnace at 570°C for 8 hours to ignite the organic material (Chapman & Pratt, 1961). The samples were cooled in a desiccator and reweighed to give an ash weight. The ash fraction was calculated as a percentage of the oven dry mass (Equation 2.3). The ash content provided an estimate of the organic matter fraction in the litter (Equation 2.4).

Equation 2.3

$$\% \text{ ash} = \text{ash weight} / \text{initial weight (OD)} \times 100$$

Equation 2.4

$$\% \text{ organic matter} = 100 - \% \text{ ash}$$

The derived values of moisture and ash were used to define parameters in subsequent experiments. All weights were recorded on oven dry (OD) and/or ash free (AF) basis.

The pH of each litter sample was measured from a suspension of sample (0.5g) and water (10mls). The suspension was placed on an orbital shaker for 2 minutes (oscillations approximately $100.min^{-1}$). The suspended material was allowed to settle and the pH measured using a Solstat EPM-610 combined electrode pH meter.

2.3.3 Mass, size, length and water retention of seeds

The mean seed mass, length and width was determined for each seed population. 100 randomly selected seeds were weighed on an analytical balance. The recorded mass was divided by 100 to provide the mean individual seed mass. The physical dimensions (length and width) of 50 randomly selected seeds from each seed population was measured using a precision venier calliper.

The potential of seeds to retain moisture within and on the seed surface was determined for each test species following a method adapted from Chadwick *et al* (1998). Weighed air dry whole seeds (2.0-10.0 g) and 50 ml of double deionised water (DDW) were placed in pre-weighed plastic containers. The containers were left for 48 hours at room temperature (20 ± 2°C). The samples were shaken occasionally to ensure all seed surfaces were in contact with the DDW. After the prescribed saturation period, gauze was placed over the lid of the containers to prevent the loss of seed fragments, and the excess water was removed. The containers were then reweighed to give an estimate of seed water retention. The mass of the moisture retained within and on the seeds' surfaces was determined as a percentage of initial seed air dry mass (Equation 2.5).

Equation 2.5

$$\% \text{ water retention} = [\,(\text{seed (WR)} - \text{seed (AD)})\,/\,\text{seed (AD)}\,] \times 100$$

Note:
AD = air dry.
WR = seed mass and moisture retained on or within the seed after 48 hours soaking in DDW.

2.3.4 Apportioning of mass and nitrogen between the seed coat and embryo-endosperm fractions

The seed coat and embryo-endosperm of 10-20 seeds of each species was quantitatively separated to determine the mass and nitrogen allocation between the two seed fractions. *Q. robur, T. aestivum, H. vulgare, N. solandri* and *N. fusca* seed fractions were carefully separated with tweezers and forceps. The seeds of *P. radiata, P. contorta, U. europaeus, C. scoparius* and *S. microphylla* were germinated following the procedures indicated in Table 2.2 to assist in the separation of the seed coat from the embryo-endosperm fraction. The seeds were air dried immediately on the emergence of the seed radical or a split in the seed testa. The seed coat and embryo-endosperm were carefully separated by hand (Jinadasa, 2000). The two seed components were placed in separate pre-weighed borosilicate glass tubes and oven dried at 105°C for 15 hours. The tubes were cooled in a desiccator and weighed on an analytical balance. The proportional allocation of seed mass to seed coat and embryo-endosperm fractions were determined using Equation 2.6 and Equation 2.7 respectively. The measured values of seed mass allocation between seed fractions were used to calculate embryo-endosperm : seed coat ratios.

Equation 2.6

Seed coat fraction (%) = (seed coat mass (OD) / total seed mass (OD)) x 100

Equation 2.7

Embryo-endosperm fraction (%) =
(seed embryo-endosperm mass (OD) / total seed mass (OD) x 100

The seed coat and embryo-endosperm fractions were ground separately with a mortar and pestle. The nitrogen (Kjeldahl) content of each homogenised fraction was quantified using the method described in Section 2.3.6. Duplicate nitrogen determinations were carried out for each seed fraction to reduce experimental error.

The results of *N. solandri* and *N. fusca* were excluded from this experiment due to the difficulty in quantitatively separating and measuring the seed coat and the embryo-endosperm fractions.

2.3.5 Lipid content

The determination of lipid content was measured twice for each seed species. Known quantities of samples (0.5-1.0 g OD) were added to a pre-weighed 50 ml borosilicate glass centrifuge tube. Distilled hexane (approximately 40 ml) was added to each tube. The tubes were sealed with a rubber stopper to prevent solvent evaporation. The samples were briefly shaken by hand (approximately two minutes) four times over a 48-hour period. The original hexane was decanted and an additional 20 ml of hexane added. The samples were shaken again. When the particulate matter settled, the solvent was decanted and the tube oven dried at 105°C for 15 hours. The percentage of lipids was determined by the mass loss that incurred through the removal of lipids (Equation 2.8).

Equation 2.8

% lipid = [(initial OD mass - final OD mass) / initial OD mass] x 100

2.3.6 Nitrogen (Kjeldahl) content

The total nitrogen content of each litter sample was determined using a semimicro-Kjedahl method adapted from Bremner (1965). Organic nitrogen was determined by the digestion of approximately 50 mg of ground litter samples (< 2mm) with concentrated sulphuric acid and a mercury catalyst as described by Bremner and Mulvaney (1982). The resultant digestion was distilled with 10M NaOH and thiosulphate alkali to liberate ammonium. The distillate was collected in boric acid indicator solution and the ammonium content titrimetrically determined with a standard 0.025M H_2SO_4 solution. The quantity of ammonium detected was converted to a percentage value of initial OD material using Equation 2.9 and Equation 2.10. All nitrogen determinations were replicated a minimum of three times for each seed sample.

Equation 2.9

Mass-Nitrogen (mg) = [(sample titre – reagent titre) x 70] / 1000

Note:
1 ml of 0.025M H_2SO_4 is required to titrate 70 μg of ammonium-N in a distillate solution.

Equation 2.10

% total - N= (Mass-N (mg) / Mass digested sample (mg)) x 100

Note:
The mass of initial digested sample is measured on an oven dry basis.

2.3.7 Characterisation of organic forms of nitrogen

The organic forms of nitrogen were identified by reflux acid hydrolysis of seed samples, with 6 N HCl for 24 hours in an oil bath at 110°C. Each sample contained approximately 10mg of nitrogen. The methodology used to quantify nitrogen fractions in the hydrolysates and acid insoluble residue is detailed in Table 2.3 and followed the techniques described by Stevenson (1982).

All nitrogen forms were converted to ammonia and steam distilled under alkaline conditions. The resulting distillate was collected in 5mL of boric acid indicator solution and titrated against a standard 0.025 M H_2SO_4 solution.

Table 2.3 Methodology to quantify organic forms of nitrogen.

Nitrogen form	Methodology
α-amino acid-N	Determined by ninhydrin-ammonium method and steam distillation with phosphate borate buffer.
Hexosamine-N	Steam distillation with phosphate borate buffer.
Ammonium-N	Steam distillation with MgO.
Hydrolysable unknown-N	Hydrolysable N not accounted for as amino acids, hexosamines, or ammonium, determined by semimicro-Kjeldahl method.
Acids insoluble unidentified-N	N remaining in sample following acid reflux hydrolysis, determined by semimicro-Kjeldahl method.
Acid soluble-N	Total N in hydrolysate determined by semimicro-Kjedahl method.

2.3.8 Oxidisable organic carbon

The total oxidisable carbon content of each litter substrate was determined using the Walkley-Black method following the technique described by Hesse (1971). Approximately 50 mg of litter sample (AD) was placed in a 500 ml wide mouth Erlenmeyer flasks. The sample in each flask was digested by the addition of 10 ml of 0.167 mol L^{-1} $K_2Cr_2O_7$ and 20 ml of concentrated H_2SO_4. The flask and contents were swirled rapidly to mix the sample and the reagents. 200 ml DDW and 0.5 ml of 0.16% w/v (aqueous) barium diphenylamine sulphonate was added to the flasks. The oxidisable carbon content was titrimetrically determined with 0.503 mol L^{-1} ammonium iron (II) sulphate. This procedure was repeated twice for each litter type and species.

In the Walkley-Black method the sample is subject to incomplete carbon oxidation. As a consequence, a correction factor must be determined (Sollins *et al*, 1999). The correction

factor was calculated by analysing the oxidisable carbon content in cellulose, assuming a 44.4% carbon content (Krässig *et al*, 1996). The correction factor was applied to each sample to account for the undetected carbon with the Walkley-Black method. The total oxidisable organic carbon as a percentage of sample mass (OD) was determined using Equation 2.11 and Equation 2.12.

Equation 2.11

Oxidisable organic carbon = (reagent titre - sample titre) x 0.003 x 100 x M x F

M = 0.503 mol L^{-1} (molarity of ammonium iron (II) sulphate).
F = correction factor of 1.21.

Equation 2.12

% C = (Oxidisable organic carbon C / sample mass (OD)) x 100

The mean carbon content and nitrogen (Kjeldahl) content (Section 2.3.6) was used to determine the carbon to nitrogen ratio of litter samples.

2.3.9 Water-soluble substances

2.3.9.1 Introduction

Soluble material is leached from the plant throughout its life via the action of rainfall, through-fall and stem flow. The quantity of leachate is governed by the accessibility of water to the substrates (Tukey, 1970) and the edaphic conditions (Swift *et al,* 1979). The release of water-soluble substances (WSS) represents a readily available source of nutrients and energy to the soil microflora that can be utilised for metabolism or resynthesis of microbial tissue (Williams & Gray, 1974; Mason, 1977). WSS were used as an indicator of potential leachable compounds. Seed leachates were extracted from each species following treatments that altered the seed surface. The resultant extractives were assayed for the concentration of reducing sugars, α-amino acids-N, ammonium-N and total nitrogen content.

The presence of inhibitory (Kremer, 1986b) and stimulatory (Harman, 1983; Rolston *et al*, 1991) compounds within seed aqueous exudates have been hypothesised to influence the presence and interactions of microbial populations. The potential anti-microbial actions of seed water-soluble extracts were investigated. Seed leachates were inoculated with soil microorganisms to investigate any net inhibitory action on microbial activity.

2.3.9.2 Extraction of water-soluble substance (WSS)

Extraction of water-soluble substances (WSS) was carried out on all species after altering the physical integrity of the seed coat. The seeds were either left whole; scarified with a scalpel; heated in an autoclave at 121°C for 20 minutes; or ground (1 mm) using a Culotti Mill. Insect damaged seeds of *U. europaeus* and *S. microphylla* were also analysed for WSS. 1.0 g of seed material and 30 ml of DDW was placed into a pre-weighed 50 ml borosilicate glass centrifuge tube. The tube was sealed with polyethylene and left for 48 hours at 5°C to minimise microbial growth. Water-soluble substances (WSS) were collected by vacuum filtration through Whatmans GF/A glass fibre filter paper (Walker, 1994). The leachate obtained from this first extraction was used in subsequent analysis of reducing sugars, α-amino acids-N, and ammonium-N concentrations. The remaining leachate from the first extraction was used in assays of microbial activity in WSS.

The 48 hour water extraction process was repeated 4 times to ensure the removal of all WSS. The remaining solid material was oven dried at 105°C to determine the mass of water insoluble substances (WIS). The mass of WIS (OD) was deducted from the total sample mass (OD) prior to water extraction to calculate WSS mass (Equation 2.13).

Equation 2.13

$$WSS\ (OD) = initial\ sample\ weight\ (OD) - WIS\ mass\ (OD)$$

Note:
WSS = water soluble substances.
WIS = water insoluble substances.

The remaining OD material was ground using a mortar and pestle. Total nitrogen (Kjeldahl) was determined for the WIS fraction and the control (unleached) material (Section 2.3.6). The WIS-nitrogen was deducted from the initial nitrogen in the control material to determine the nitrogen content of the water-soluble fraction (Equation 2.14).

Equation 2.14

$$WSS\text{-}N = Total\text{-}N - WIS\text{-}N$$

Note:
WSS = water soluble substances.
WIS = water insoluble substances.

2.3.9.3 Reducing sugars

The concentration of reducing sugars present in the water-soluble extract was determined colorimetrically using the hydroxybenzoic acid hydroxide (HBH) method (Lever, 1973). A standard curve was constructed using a dilution series of glucose made up to a final volume of 2 ml. 5 ml of HBH was added to 20 µl of each glucose concentration. The content of each tube was mixed thoroughly and placed in a boiling water bath for 5 minutes. The absorbance was read at 430 nm using a spectrophotometer (Novaspec ® II). These values were used to plot absorbance (A 430 nm) against sugar concentration (mg ml^{-1}) thereby producing a calibration curve.

WSS (20 µl) extracts from each of the seed samples were assayed for reducing sugar concentration. 5 mls HBH was added to each extract. The tubes were placed in a boiling water bath for 5 minutes and the subsequent absorbance measured. The calibration curve was used to estimate the concentration of sugar present in each seed extract.

2.3.9.4 α-Amino acid-N

The ninhydrin-ammonium method (Section 2.3.7) was applied to 5 ml of each seed extract to determine α-amino acid-N in water-soluble leachates. The concentration of α-amino acids-N was quantified using Equation 2.15.

Equation 2.15

$$\alpha\text{-amino acid-N } (\mu g) = (\text{sample titre} - \text{reagent titre}) \times 70$$

2.3.9.5 Ammonium-N

Ammonium was quantified by distillation with approximately 200 mg of magnesium oxide (Section 2.3.7). The concentration of ammonium-N in the water-soluble extracts were quantified using Equation 2.16.

Equation 2.16

$$\text{ammonium-N} = (\text{sample titre} - \text{reagent control}) \times 70$$

2.3.9.6 Microbial activity in seed leachates

To investigate microbial activity, 6-8 ml of WSS from the first extraction was added to two 250ml Erlenmeyer flasks. The first flask contained 100 µl of Ilam soil inoculum and was covered with polythene to prevent moisture loss. The soil inoculum was prepared by thoroughly mixing 9 ml of DDW and 1 g of Ilam soil from the University of Canterbury grounds. The particulate matter was left to settle and the clear liquid was used to inoculate the WSS. The second flask (control) contained 100 µl of soil inoculum and 0.5 ml of chloroform to prevent microbial growth. A rubber stopper in the mouth of the flask was used to prevent chloroform evaporation. The flasks were placed on an orbital shaker (oscillations approximately 100 min^{-1}) and incubated at room temperature (20 ± 2°C) for 7 days. After this period, the microbial growth was qualified on an arbitrary scale by visually determining the colour, opacity and presence of microbial flocs (see Table 2.4). The pH was recorded before and after incubation using a Solstat EPM-610 combined electrode pH meter.

20 µl aliquot of each water-soluble seed extract was plated out on Plate Count Media. The plates were incubated at 25°C for 24 hours. Observations were made of colony characteristics and the dominant bacteria colonies were examined microscopically (Olympus BH-2).

Table 2.4 Description of microbial growth ranks in seed leachates (0 - ++++).

Rank	Microbial growth
0	Leachate clear.
+	Leachate slightly turbid.
++	Leachate turbid; <5 microbial flocs.
+++	Leachate turbid; 5-15 microbial flocs; minimal growth on glass.
++++	Leachate turbid; >15 microbial flocs; extensive growth on glass.

2.4 MICROBIAL DECOMPOSITION IN MICROCOSMS

2.4.1 Introduction

Various techniques have been used by researchers to investigate the decomposition of litter. These methods include; i) estimation of litter mass loss *in situ* or in microcosms under controlled conditions; ii) incorporating and apportioning of isotopes, such as ^{14}C and ^{15}N into the decomposition system; iii) measurements of carbon dioxide evolution to quantify net heterotrophic activity; and iv) chemical analysis of total nutrient concentration or net nutrient mineralisation.

Microcosms are, in theory, representative of the natural environment (Taylor & Parkinson, 1988). Microcosms therefore provide a simple method to observe and precisely measure decomposition under controlled conditions. Researchers are able to manipulate individual or multiple factors to investigate their effect on the decomposition process.

Seed samples of the 10 test species were decomposed in the presence of mixed soil microbial populations. Substrate quality, physico-chemical environment and microbial communities were manipulated to investigate their potential roles in seed degradation. Weight loss and net nitrogen mineralisation were used as indicators of microbial decomposition. The procedure of microcosm set up and analysis were analogous to that of Greenfield (1999). A summary of the microcosm experiments and the various treatments are listed in Table 2.5.

Table 2.5 Summary of substrate quality, environmental conditions and microbial community in each microcosm experiment.

EXPT		SQ		ENV			MO	SPECIES
Title	*Section*	*Physical*	*Chemical*	*Temp (°C)*	*Moist (%)*	*Light (Lx)*	*Soil inoculum*	
Constant temperature	2.4.6.1	Whole	None	20	60-70	190	ISI	TA, HV, PR, PC, NF, NS, CS, UE, QR, SM
Microbial decomposition of whole seeds	2.4.4.1	Whole	None	5-15	60-70	None	ISI	TA, HV, PR, PC, NF, NS, CS, UE, QR, SM
Variation in seed coat integrity	2.4.4.2	Scarify	None	5-15	60-70	None	ISI	TA, HV, PR, PC, NF, NS, CS, UE, QR, SM
Variation in seed coat integrity	2.4.4.2	Autoclave	Autoclave	5-15	60-70	None	ISI	TA, HV, PR, PC, NF, NS, CS, UE, QR, SM
Variation in seed coat integrity	2.4.4.2	Ground	None	5-15	60-70	None	ISI	TA, PR, NF, NS, UE, SM
Litter mixture	2.4.4.5	Ground	Litter mixture	5-15	60-70	None	ISI	NS, NF, PR, UE
Native soil microbes	2.4.5.2	Whole	None	5-15	60-70	None	ISI, HSI, DSI, LSI	PR, NS, CS
Removal of labile compounds	2.4.4.3	Whole	Labile-free	5-15	60-70	None	ISI	TA, HV, PR, PC, NF, NS, CS, UE, QR, SM
Endophytes	2.4.5.1	Whole	None	5-15	60-70	None	None	PR, PC
Wet-and-dry cycle	2.4.6.3	Whole	None	20	10-70	190	ISI	PR, PC, NF, NS, CS, UE, SM
Freeze-and-thaw cycle	2.4.6.4	Whole	None	-4-+20	60-70	None -190	ISI	PR, PC, NF, NS, CS, UE, SM
Insect damaged seeds	2.4.4.4	Insect damaged	None	5-15	60-70	None	ISI	SM, UE
Immature seeds	2.4.4.4	Immature	Immature	5-15	60-70	None	ISI	UE

Key:

SQ = substrate quality, ENV = environmental conditions, MO = microbial community, Temp = temperature, Moist = moisture.

Temperature: 5-15 = cycling between 5°C for 12 hours and 15°C for 12 hours, -4-+20 = cycling between –4°C and 20°C.

Moisture: 60-70 = moisture held constant at 60-70%, 10-70 = cycling between 60-70% moisture for 7 days and 10-20% moisture for 7 days.

Species: TA = *T. aestivum*, HV = *H. vulgare*, PR = *P. radiata*, PC = *P. contorta*, NF = *N. fusca*, NS = *N. solandri*, CS = *C. scoparius*, UE = *U. europaeus*, SM = *S. microphylla*, QR = *Q. robur*.

Microbial communities: ISI = Ilam soil inoculum, HSI = Hawdon soil inoculum, DSI = Darfield soil inoculum, LSI = Lincoln soil inoculum.

2.4.2 Weight loss microcosms (WLM)

The weight loss microcosms (WLM) used in all experiments were set up and destructively analysed using the procedures described in Sections 2.4.2.1 and 2.4.2.2. Unless otherwise stated, the mass of the seed material, moisture content, soil inoculation and destructive analysis is identical in all microcosms.

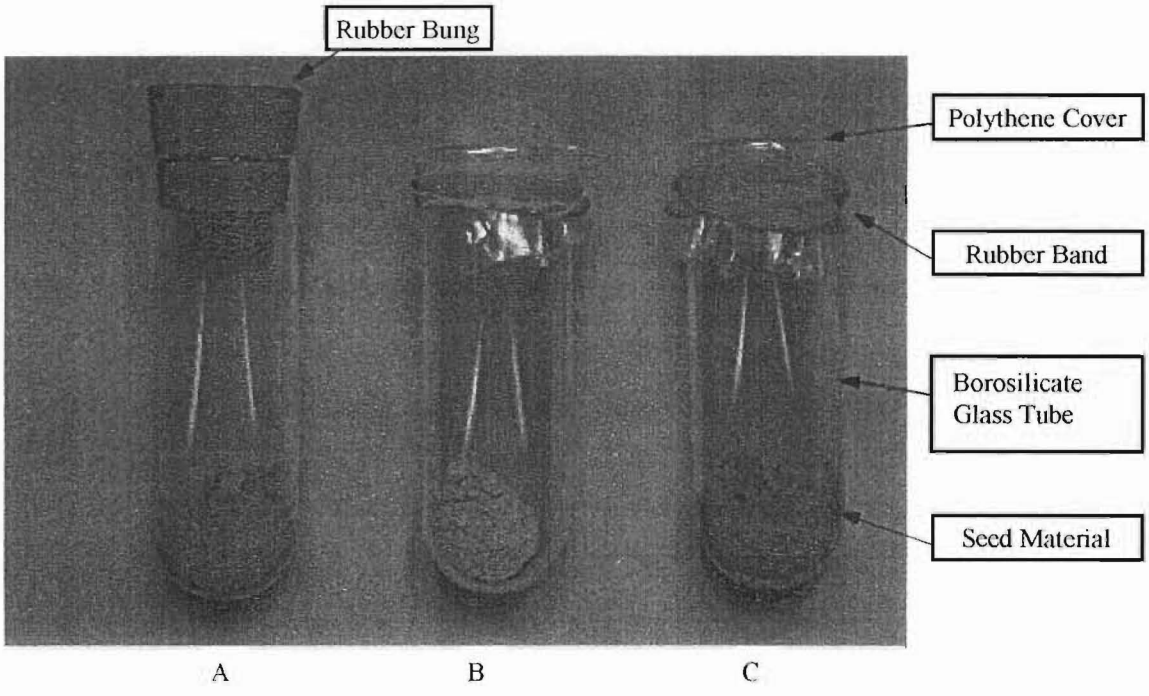
2.4.2.1 Weight loss microcosm (WLM) set up

Known weights of litter samples varying between 0.3-0.5 g (AD) were placed into pre-weighed 50 ml borosilicate glass centrifuge tubes. *Q. robur* seed material weighed between 6.0-7.0 g (AD) due to the large seed size. The seed substrates were moistened with DDW and 100 µl of soil inoculum to bring the samples to approximately 60-70% moisture content (dry weight basis). The microcosms appeared moist with no free water. The soil inoculum was prepared by mixing 9 ml of DDW and 1 g of Ilam soil. The particulate matter was left to settle and the clear liquid was used to inoculate the microcosms. Unless otherwise stated, this inoculation procedure was used in all microcosm experiments.

Where possible the samples were evenly spread around the bottom 2 cm of the tube. The tubes were covered with polythene to allow gas diffusion but prevent moisture loss. Two controls were set up for each treatment. The first control contained sample, soil inoculum, moisture and chloroform to measure any 'decomposition' in the absence of microorganisms. The second contained the litter sample, soil inoculum and no moisture to determine the role of water in decomposition and account for any seed respiration contributing to weight loss. The experimental and control microcosm set up is shown in Figure 2.2.

The microcosms were checked regularly (approximately every 2 weeks) to ensure adequate moisture was present and to harvest any seedlings. The quantity of water added varied between species and within replicates, due to variation in seed imbibition and microbial growth. Seed germination and the associated seedling respiration in the microcosm experiments, potentially reduced the power of weight loss measurement to quantify decomposition. If a seed germinated, the radical was detached from the seed before replacing it back into the microcosm.

Figure 2.2 Weight loss microcosm set up A) control microcosm containing litter, moisture, soil inoculum and chloroform; B) control microcosm containing litter, soil inoculum, and no moisture; C) experimental microcosm containing litter, soil inoculum, and moisture.



2.4.2.2 Weight loss microcosm (WLM) analysis

Weight loss microcosms (WLM) were visually observed and scored for microbial growth and physical status of the seeds on an arbitrary 4 star scale as shown in Table 2.6. The microbial description is accompanied by a percentage range of seeds covered with microbial growth within the microcosm.

Table 2.6 The ranks of microbial growth and seed physical status.

Rank	Microbial growth and physical status of substrate
0	No microbial growth (0%)
+	Minimal microbial growth (1-10%)
++	Seed discolouration and microbial growth (11-50%)
+++	Extensive seed discolouration and microbial growth (51-90%)
++++	Extensive discolouration and extensive microbial growth (91-100%)

Following incubation, the tubes were placed in a 105°C oven for 24 hours. The tubes were cooled in a desiccator and weighed on an analytical balance to give a final OD weight. This figure was then deducted from the initial OD weight of the sample and converted to a percentage using Equation 2.17. All calculations were performed on an oven dry, ash free

basis. The mass loss incurred in the control microcosms was deducted from the final mass loss (%) to account for seed respiration. In all cases this was less than 3%.

Equation 2.17

% mass loss = (initial mass (OD) - final mass (OD) / initial mass (OD)) x 100

The mass loss after 180 days was used to calculate the decay rate constants (k) assuming a curvilinear response of mass loss over time (Olson, 1963). The rate of decomposition was calculated using an exponential decay model (Equation 2.18).

Equation 2.18

$$X_t = X_0 e^{-kt}$$

Note:
X_t = seed mass at time t.
X_0 = initial seed mass.

2.4.3 Nitrogen mineralisation microcosms (NMM)

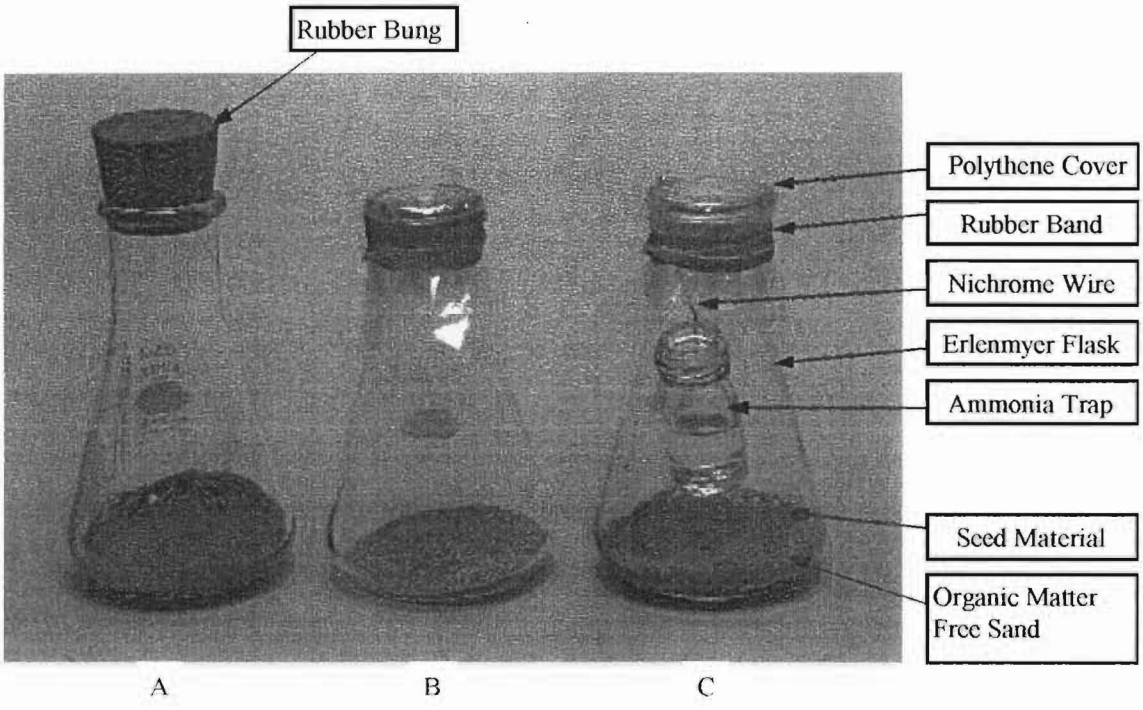
The nitrogen mineralisation microcosms (NMM) used in all experiments were set up and destructively analysed following the procedure described in Sections 2.4.3.1 and 2.4.3.2. Unless otherwise stated the mass of the seed material, moisture content, soil inoculation and destructive analysis was identical in all microcosms.

2.4.3.1 Nitrogen mineralisation microcosm (NMM) set up

Known amounts of sample (0.3-0.5 g (AD)), with the exception of *Q. robur* (6.0-7.0g (AD)) and 5g of ignited sand were placed in 250 ml Erlenmeyer flasks DDW and 100 µl of soil inoculum (refer to Section 2.4.2.1) were added to adjust the microcosms to 30-40% moisture content (dry weight basis). The sand and litter material appeared moist, however no free water was present. Ammonia traps containing 2 mls of 2M H_2SO_4 were suspended in the flask necks with nichrome wire. The flasks were covered with polythene to allow gas exchange but prevent moisture loss. Two controls were set up for each treatment to provide an estimate of the mineral nitrogen present in the absence of microbial decomposition. The first control contained litter sample, soil inoculum, moisture and chloroform. The second control contained litter sample, soil inoculum and no moisture. The experimental and control

microcosms set up is shown in Figure 2.3. The microcosms were examined at fortnight intervals to ensure adequate moisture and to harvest any germinated seeds.

Figure 2.3 Nitrogen mineralisation microcosm set up. A) control microcosm containing ignited sand, litter, moisture, soil inoculum and chloroform; B) control microcosm containing ignited sand, litter, soil inoculum, and no moisture; C) experimental microcosm containing ignited sand, litter, soil inoculum, moisture and an ammonia trap.



2.4.3.2 Nitrogen mineralisation microcosm (NMM) analysis

The microcosms were visually examined for microbial growth and the physical status of the seeds. Qualitative observations were recorded on a four star arbitrary scale (0 - ++++) summarised in Table 2.6. 10ml of DDW was added to each flask. The flasks were placed on an orbital shaker (oscillations approximately 100.min^{-1} for 2 minutes and the pH of the resulting suspensions were measured using a Solstat EPM-610 combined electrode.

The net nitrogen mineralisation in each microcosm was determined as the sum of the mineral nitrogen in the microcosm and the ammonia trap. The mineral nitrogen present in microcosms was determined by KCl extraction. 30 ml of 2M KCl was added to each flask and left for approximately 20 hours with occasional hand shaking. 10-20 ml aliquots of the KCl suspension were analysed for ammonium-N and nitrate-N by steam distillation with

approximately 200 mg of MgO and 100 mg of Devarda's alloy respectively, as described by Keeney and Nelson (1982). The nitrogen in the distillate was titrimetrically determined with a standard 0.025M H_2SO_4 solution. The percentage of sample nitrogen mineralised was calculated using Equation 2.19 and Equation 2.20.

Equation 2.19

(ammonium + nitrate)-N(mg) = (sample titre-reagent titre) x 70) / 1000

Equation 2.20

% total-N mineralised = (ammonium + nitrate)-N(mg) / initial sample-N(mg) X 100

The content of the ammonia trap was transferred into 100 ml Kjeldhal flask and steam distilled with 3 ml of 10 M NaOH. The distillate was collected in boric acid indicator solution and titrated against a standard 0.025M H_2SO_4 solution. The total ammonium-N in the ammonia trap was determined using Equations 2.21 and 2.22.

Equation 2.21

Ammonium-N (mg) = (sample titre –reagent titre) X 70 / 1000

Equation 2.22

% total-N = (ammonium-N(mg) / initial-N(mg)) X 100

The net nitrogen mineralisation was calculated by combining the amount of ammonium-N present in the ammonia trap and the quantity of nitrate-N and ammonium-N present in the sand. This figure was deduced from the mineral nitrogen present in control microcosms. The mineral nitrogen present in the seed material at day 0 did not exceed 1% in all test species.

2.4.4 Variation in substrate quality

2.4.4.1 Microbial decomposition of whole seeds

2.4.4.1.1 Introduction

Seeds of the ten test species were incubated in the presence of moisture and soil microbes to investigate if the seed substrates were microbially decomposed.

2.4.4.1.2 Methodology

Weight loss microcosms (WLM) and nitrogen mineralisation microcosms (NMM) containing whole seeds were set up and analysed for each species following the procedures in Sections 2.4.2 and 2.4.3 respectively. Microcosms contained between 0.4-0.5 g of whole seeds, with the exception of *Q. robur* microcosms, which contained 6.0-7.0 g. Three sets of triplicate microcosms were set up and incubated for 30, 90, and 180 days allowing a time series of decomposition to be constructed. The microcosms were incubated in a growth chamber with no light and the temperature cycling between 5 ± 2°C for 12 hours and 15 C ± 2°C for 12 hours. These temperatures approximate the diurnal temperature cycles.

2.4.4.2 Variation in seed coat integrity

2.4.4.2.1 Introduction

The seed coat functions as a protective barrier between the embryo and the external environment. Changes in the integrity of the seed coat may alter the effectiveness of the seed coat and increases the surface exposed to microbial catabolic activities. Changes in the seed coat integrity and its influence on the rate of seed degradation was investigated by decomposing seeds subjected to scarification, heat and ground treatments.

2.4.4.2.2 Methodology

The physical nature of the seed was altered using 3 treatments: scarification, ground and heat (Table 2.7).

Table 2.7 Treatments altering the seed coat integrity.

Seed Treatment	Methodology	Analogous treatments in nature
Whole	Unmodified	Intact seed coat.
Scarification	2-15 mm incision in seed coat with razor blade.	Scarification through wet-and-dry cycles, freeze-and-thaw cycles, microbial degradation, mechanical abrasion, hydrolysis in animal digestive tract.
Ground	Culotti Mill through a 1mm² filter.	Macerating and comminutive activity of invertebrates and vertebrates.
Heat	Autoclave for 20 minutes at 121°C in sealed containers.	None, necessary but unrealistic control. Autoclaving denatures proteins and alters cell membranes.

The extreme temperature and pressure of the autoclave treatment subjected the seeds to conditions not present in the natural environment. Autoclaving potentially causes irreversible damage to membranes and denatures enzymes of living tissue. Despite the 'artificial' nature of this treatment, it was selected as the universal way to kill the seeds of all test species.

WLM and NMM were set up and analysed according to the procedures outlined in Sections 2.4.2 and 2.4.3. Each microcosm contained 0.4-0.5 g of seed material for all species with the exception of *Q. robur* microcosms, which contained 6.0-7.0 g of seed material. Scarification and heat treatment was applied to all test species. Only the seeds of *T. aestivum*, *P. radiata*, *U. europaeus*, *N. fusca*, *N. solandri* and *S. microphylla* were subjected to ground treatments.

Three series of WLM and NMM were set up with 30, 90 and 180 day incubation periods for each seed treatment. The scarification and ground treatments were set up in triplicate whereas the heat treatment was only duplicated at each incubation period. All the microcosms were incubated in a growth chamber with temperature cycling every 12 hours between 5 ± 2 °C and 15 ± 2 °C in the absence of light.

2.4.4.3 Removal of labile compounds

2.4.4.3.1 Introduction

When seeds are placed in water, the membranes become leaky as they reconstitute. During this period (before the membranes are repaired) electrolytes, proteins and low molecular

weight compounds may be lost from the seeds (Bewley & Black, 1978; Mason, 1977). The loss of these labile compounds represents a source of readily available energy to microorganisms (Lynch, 1978). The initial release of water-soluble compounds from a concentration of seeds may represent a flush of nutrients, followed by a concomitant flush of microbial growth, exposing the seeds to pressures uncharacteristic of natural conditions. This was investigated by decomposing whole seeds following the removal of water-soluble substances.

2.4.4.3.2 Methodology

Known quantities of whole seeds (0.4-7.0g) were soaked in DDW for 48 hours. The labile free seeds were incorporated into NMM (Section 2.4.3.1). Additional DDW and 100 µl of Ilam soil inoculum was added until the microcosms appeared wet but with no free water (approximately30-40% moisture content). Two sets of duplicate NMM were set up for each species and destructively analysed after 30 or 90 days incubation in a dark growth chamber with temperatures cycling between 5-15 ± 2 °C over a 24 hour period (Section 2.4.3.2).

2.4.4.4 Insect-damaged and immature seeds

2.4.4.4.1 Introduction

Insect and microbe interactions can influence the deterioration of seeds (Mills, 1983). The feeding activity of insects will make seed substrates available to microorganisms either directly, by rupturing the seed coat and making seeds vulnerable to microbial invasion, or indirectly, via the insects' excretion products (Janzen, 1977).

Sophora microphylla suffers up to 28% predation from the moth larvae *Stathmopoda aposema*. The larval invasion causes fatal damage to the embryo and cotyledons (Webb, 1993). *Ulex europaeus* is parasitised by the gorse seed weevil *Apion ulicis* (Hill *et al*, 1991). The potential decomposition of insect damaged seeds of *S. microphylla* and *U. europaeus* and the immature seeds of *U. europaeus* were investigated.

2.4.4.4.2 Methodology

Green coloration was assumed to be indicative of seed immaturity in *U. europaeus*. Insect predated seeds of *S. microphylla* and *U. europaeus* were excised of insect frass external to the seed.

WLM and NMM containing 0.4-0.5 g of insect damaged seeds from *S. microphylla* and *U. europaeus* and immature seeds of *U. europaeus* were set up and analysed according to procedures described in Sections 2.4.2 and 2.4.3. Three sets of WLM and NMM were set up in triplicate and incubated for 30, 90, or 180 days in a dark growth chamber at temperatures cycling 5-15 ± 2 °C over a 24 hour period.

2.4.4.5 Litter mixtures

2.4.4.5.1 Introduction

The presence of multiple litter types may either enhance (Taylor *et al*, 1989b; Briones & Ineson, 1996, McTiernan *et al* 1997; Chadwick *et al*, 1998) or retard (Finzi & Canham, 1998; Bardgett & Shine, 1999; Robinson *et al*, 1999) the components' decomposition rate. Combinations of intra-species litter types were decomposed together to investigate potential interactions between litter components. Decay characteristics of litter decomposing in isolation were compared with the decomposition of litter mixtures to investigate any non-additive effects.

2.4.4.5.2 Methodology

Litter combination experiments were carried out on ground (1 mm) seed, leaf and twig (<3 mm) litter of *N. solandri, N. fusca, P. radiata* and *U. europeaus*. WLM (duplicate) and NMM (triplicate) were set up containing a fixed quantity of litter substrate (0.3-0.5 g). Each microcosm contained either a single litter type or equal proportions of litter combinations as shown in Table 2.8. The litter was thoroughly mixed to ensure maximum contact between the litter components for each litter mixture. The set up of the MLM and NMM is detailed in Sections 2.4.2.1 and 2.4.3.1 respectively. The microcosms were incubated in a dark growth chamber with the temperature cycling between 5-15 ± 2°C over a 24 hour period. Three groups of microcosms were set up and incubated for 30, 90 or 180 days, allowing a time series of decomposition to be constructed.

Table 2.8 Litter mixtures in microcosms.

Litter type	Species			
	P. radiata	*U. europaeus*	*N. solandri*	*N. fusca*
Seed litter (S)	+	+	+	+
Leaf litter (L)	+	+	+	+
Twig litter (T)	-	-	+	+
S + L	+	+	+	+
S + T	-	-	+	+
L + T	-	-	+	+
S + L + T	-	-	+	+

Note

- + = species subjected to litter combination treatment; - = species not subjected to litter combination treatment.
- S = seed, L = leaf, T = twig.

The WLM and NMM were destructively analysed following their respective incubation periods (Section 2.4.2.2 and Section 2.4.3.2). The results from the microcosms containing pure litter types were used to predict the mass loss and the nitrogen mineralised from the litter combinations (Equation 2.23 and Equation 2.24). The predicted values were compared with actual values obtained from microcosms containing litter mixtures to investigate any non-additive effects.

Equation 2.23

$$EXML_{ABmix} = [MA_{ABmix} \times (ML\%A_{Apure}/100)] + [MB_{ABmix} \times (ML\%B_{Bpure}/100)]$$

Note

- $EXML_{ABmix}$ = expected mass loss of A and B litter in mixture.
- MA_{ABmix} = mass of litter A in litter mixture AB.
- $ML\%A_{Apure}$ = percentage mass loss of litter A in pure litter.
- MB_{ABmix} = mass of litter B in litter mixture AB.
- $ML\%B_{Bpure}$ = percentage mass loss of litter B in pure litter.

Equation 2.24

$$EXNM_{ABmix} = [NA_{ABmix} \times (NM\%A_{Apure}/100)] + [NB_{ABmix} \times (NM\%B_{Bpure}/100)]$$

Note

- $EXNM_{ABmix}$ = expected nitrogen mineralisation from A and B litter in mixture.
- NA_{ABmix} = nitrogen mass of litter A in litter mixture AB.
- $NM\%A_{Apure}$ = percentage net nitrogen mineralisation from litter A in pure litter.
- NB_{ABmix} = nitrogen mass of litter B in litter mixture A.
- $NM\%B_{Bpure}$ = percentage net nitrogen mineralisation from litter B in pure litter.

2.4.5 Microbial community

2.4.5.1 Endophytes

2.4.5.1.1 Introduction

The microbial populations present on or within the seed are transported with the host tissue, and have the potential to colonise when conditions are favourable. By 1979, 1,500 seed borne isolates had been identified (McGee, 1983), with fungi making up 72% of those listed (Richardson, 1996). It has been hypothesised that the microbes present on the seed coat are more efficient at utilising seed substrates (van Leeuwen, 1981). The potential for seed endophytes to decompose seeds in the absence of soil microorganisms was investigated for *P. radiata* and *P. contorta.*

2.4.5.1.2 Methodology

WLM and NMM were set up containing 0.4-0.5 g of *P. radiata* and *P. contorta* whole seeds. The microcosms were set up and analysed according to the procedures outlined in Sections 2.4.2 and 2.4.3, however no soil inoculum was added in the process of hydrating the microcosms and their constituents. The microcosms were incubated in a dark growth chamber with temperatures cycling between 5-15 ± 2°C over a 24-hour period. Three series of duplicate WLM and triplicate NMM were incubated for 30, 90 or 180 days. This allowed a time series of decomposition to be constructed.

2.4.5.2 Native soil microorganisms

2.4.5.2.1 Introduction

Variation in the chemistry and physical properties of the litter substrates would presumably have selective effects on the heterotrophic community (Wardle & Lavelle, 1997). The 'importance' of microbial trophic groups will change over time as the substrate is altered through a cascade of interactions with the biotic and abiotic environment. The presence or absence of specialised microbial groups, with specific enzymatic capabilities to degrade recalcitrant materials, has the potential to influence the projected decomposition rate of substrates.

Previous experiments in this research have made an assumption of minimal interaction between the seed and native soil microorganisms. It has been assumed that the cosmopolitan Ilam soil inoculum contains congruent microbial enzymatic diversity to degrade seed substrates. The decomposition rate of seed substrates may be an artefact of the diversity of microbes present. To investigate any selective effects of Ilam soil inoculum, seeds were inoculated with soil microorganisms from sites where the species is vegetatively dominant.

2.4.5.2.2 Methodology

Soil samples were collected from the top 10 cm of the soil profile of four sites of variable land use and vegetation cover as shown in Table 2.9. The location of the sites is shown in Figure 2.1 as Site 1, Site 2 and Site 3.

Table 2.9 Soil collection sites.

Site	Collection Site	Dominant vegetation	Land use	Soil Inoculum
1	Hawdon Valley	*N. solandri*	Beech forest	Hawdon (HSI)
2	Lincoln	*C. scoparius*	Arable pasture	Lincoln (LSI)
3	Darfield	*P. radiata*	Pine plantation	Darfield (DSI)

Note
HSI = Hawdon soil inoculum, LSI = Lincoln soil inoculum, DSI = Darfeild soil inoculum.

A soil inoculum was made for each site using a fresh soil sample. The soil inoculum was prepared by thoroughly mixing 9 ml of DDW and 1 g of soil. The particulate matter was left

to settle and the clear liquid was used to inoculate microcosms. Duplicate WLM and triplicate NMM containing whole seeds of *N. solandri*, *P. radiata* and *C. scoparius* were set up using soil inoculums HSI, DSI and LSI respectively. The microcosms were set up using the procedures described in Sections 2.4.2.1 and 2.4.3.1. However, soil inoculation was carried out using native soil microorganisms. The microcosms were incubated in a dark growth chamber at temperatures cycling between 5-15 ± 2°C over a 24 hour period. Three series of triplicate microcosms for each species were set up and incubated for 30, 90, or 180 days. The WLM and NMM were analysed after their assigned incubation periods using the procedures outlined in Sections 2.4.2.2 and 2.4.3.2 respectively.

2.4.6 Temperature and moisture variation

The physico-chemical environment is a key variable altering the decomposition process through its interactions with the substrate quality and soil microorganisms (Swift *et al*, 1979). The influences of a range of temperature and moisture conditions on seed decomposition were investigated.

2.4.6.1 Constant temperature

2.4.6.1.1 *Introduction*

Temperature influences the growth and activity of soil microbes, consequently altering the rate of decomposition. Whole seeds were decomposed under optimum conditions of temperature and moisture. Experiments under these conditions provide a projected estimate of the maximum decomposition rate.

2.4.6.1.2 Methodology

WLM and NMM containing 0.5-7.0 g of whole seeds of the 10 test species were set up and analysed using the procedures outlined in Sections 2.4.2. and 2.4.3. Duplicate microcosms of each species were incubated at room temperature (20 ± 2°C). The light regime cycled approximately 12 hours at low light and 12 hours with no light. The low light was estimated at 190 Lx using a SEKONIC Exposuremeter Flashmate L-308B11. Three sets of microcosms were set up and incubated for 30, 90 or 180 days, allowing a time series of decomposition to be constructed.

2.4.6.2 Variable temperature

2.4.6.2.1 Introduction

The soil surface is the interface between the soil and the atmosphere. As a consequence, the abiotic conditions at the surface closely reflect the atmospheric conditions. The litter in the soil surface will be exposed to diurnal and seasonal fluctuations in temperature and moisture. Seed substrates were decomposed under a continually cycling temperature regime to approximate diurnal temperature variation on the soil surface.

2.4.6.2.2 Methodology

Microcosms were incubated in a growth chamber in the absence of light. The temperature varied from 12 hours at 5 ± 2 °C and 12 hours at 15 ± 2°C. The microcosm experiments varying the substrate quality (Section 2.4.4) and the microbial community (Section 2.4.5) were conducted under this temperature regime.

2.4.6.3 Wet-and-dry cycles

2.4.6.3.1 Introduction

Seeds on the soil surface are exposed to variable conditions of moisture. Fluctuations in moisture availability may potentially effect the physical properties of the substrate and the activity of the microbial populations (Chen & Alexander, 1973; Schimel *et al*, 1999). Wet-and-dry cycles were simulated in the laboratory to investigate any potential effect on seed decomposition.

2.4.6.3.2 Methodology

WLM and NMM were set up with 0.4-0.5 g of *C. scoparius, U. europaeus, S. microhylla, P. radiata, P. contorta, N. fusca* and *N. solandri* whole seeds, following the procedures detailed in Sections 2.4.2.1 and 2.4.3.1. The microcosms were subjected to cycles of high and low moisture.

Initially the moistened microcosms were covered with polythene to prevent moisture loss. The samples were incubated for 7 days under optimum moisture conditions at room

temperature (approximately 20 ± 2°C). The light conditions within the laboratory approximated a 24-hour cycle of low light (190 Lx) and no light. The polythene covers were removed and the samples subjected to drying winds generated by a fume cupboard (approximately 10 km hr^{-1}) to increase the rate of moisture loss for a further 7 days. During the drying period the ammonia traps of NMM were removed and capped to prevent a loss of ammonia. Following the completion of the 14 day wet-and-dry cycle, the microcosms were re-moistened to the original moisture content with DDW. The samples appeared moist with no free water. The periodic drying killed all developing seedlings, therefore no harvesting of germinated seedlings was required. Three sets of duplicate WLM and duplicate NMM were subjected to 14 day wet-and-dry cycles for 30, 90 or 180 days. The WLM and NMM were analysed after their assigned incubation times according to procedures outlined in Sections 2.4.2.2 and 2.4.3.2.

2.4.6.4 Freeze-and-thaw cycles

2.4.6.4.1 Introduction

Periodic freezing and thawing events will effect both the seed substrate and the microbial community. The freezing events result in extreme temperatures and a reduction in the availability of free water to the microbial community. Fluctuations in temperature and water availability may cause the repetitive expansion and contraction of cells, consequently altering the integrity of cell membranes (Fenner, 1985). Seed substrates were subjected to repetitive freeze-and-thaw cycles to investigate their effects on seed decomposition.

2.4.6.4.2 Methodology

WLM and NMM containing 0.4-0.5g of *C. scoparius, U. europaeus, S. microhylla, P. radiata, P. contorta, N. fusca* and *N. solandri* whole seeds were set up according to the procedures outlined in Sections 2.4.2.1 and 2.4.3.1. The microcosms were subjected to periodic freezing temperatures (approximately -4°C) for between 12 and 48 hours. This freezing event was followed by a thawing period of equal length at room temperature (approximately 20 ± 2 °C). Three series of duplicate WLM and duplicate NMM were set up for each species and incubated under repetitive freeze-and-thaw conditions for 30, 90 or 180 days. The number of freeze-and-thaw cycles experienced by microcosms incubated for 30, 90 and 180 days were approximately 12, 36 and 72 respectively. No harvesting of seedlings was

required as no germination was observed for any species. The WLM and NMM were destructively analysed after their respective incubation periods according to procedures outlined in Sections 2.4.2.2 and 2.4.3.2.

2.5 *IN SITU* DECOMPOSITION OF SEEDS

2.5.1 Introduction

In situ decomposition was investigated by enclosing seeds in nylon mesh bags ('litter bags') and incorporating the sample into the litter or soil horizon. The 'litter bag' technique provides a simple and convenient method to investigate net changes in the confined litter under field conditions. Changes in seed mass and seed nitrogen dynamics were used to investigate differences in the decomposition of live and heat-killed seeds, seeds incubated at differing soil depth and seeds exposed to various soil mediums.

2.5.2 *In situ* decomposition on litter surface and within the soil

2.5.2.1 Introduction

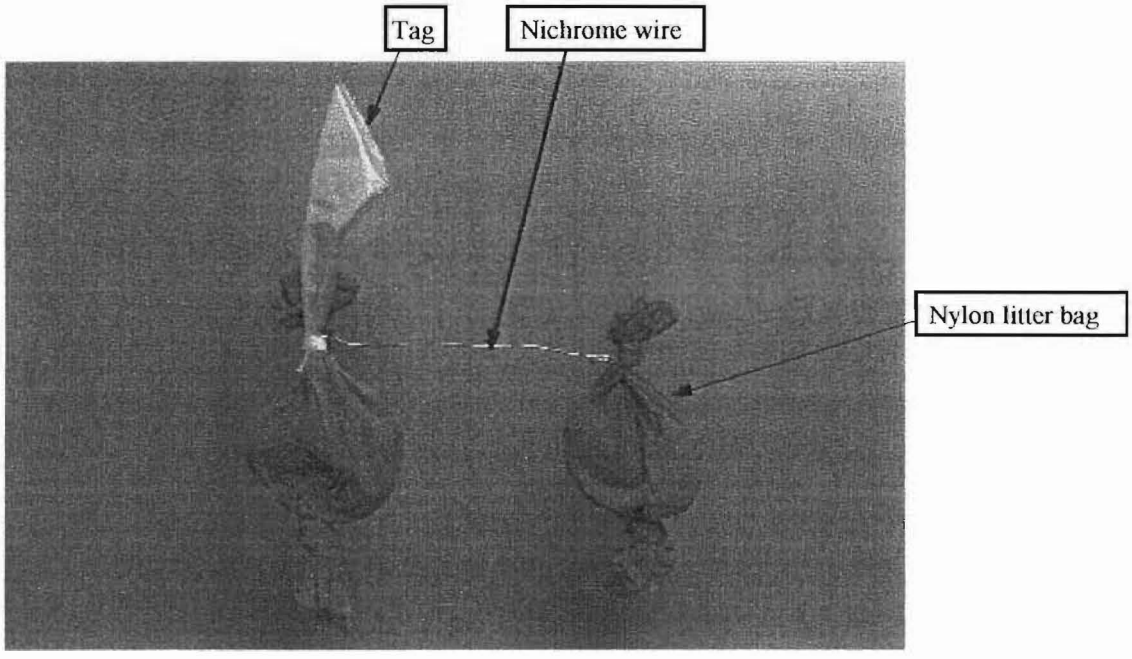
Surface soils experience marked oscillations in moisture and temperature. The amplitude of these fluctuations decreases steeply with increasing depth due to the buffering capacity of the soil. The litter on the surface and the litter buried within the soil would therefore be expected to experience different physical conditions and a concomitant variation in microbial activity (Witkamp & Olson, 1963; Swift *et al*, 1979). Seeds were confined in litter bags and incubated at soil depths of 1 cm and 10 cm to investigate the influence of depth on seed decomposition.

2.5.2.2 Methodology

Whole seeds (0.5-7.0 g) of *T. aestivum, H. vulgare, C. scoparius, U. europaeus, S. microphylla, P. radiata, P. contorta, N. fusca, N. solandri* and *Q. robur* were enclosed in pre-weighed litter bags (8 x 5 cm). The litter bags were constructed from nylon mesh (14-22 μm^2) to allow the movement of water, microorganisms and small invertebrates between the soil and the litter bag. Pairs of litter bags were connected with a 10 cm length of wire, and tagged to aid in locating the samples after incubation. The set up of litter bags is shown in Figure 2.4.

The pairs of litter bags were placed vertically in the soil, with the individual litter bags lying horizontally. Three series of duplicate pairs of litter bags were incubated in the Ilam soil on the University of Canterbury campus for 30, 90 or 180 days, allowing a time series of decomposition to be constructed. Control bags (triplicate) containing a known weight of Whatman GF/A filter paper were incubated in the soil to account for any decomposition of the nylon material and soil contamination. All the litter bags were buried within a $2m^2$ area, shown in Figure 2.5.

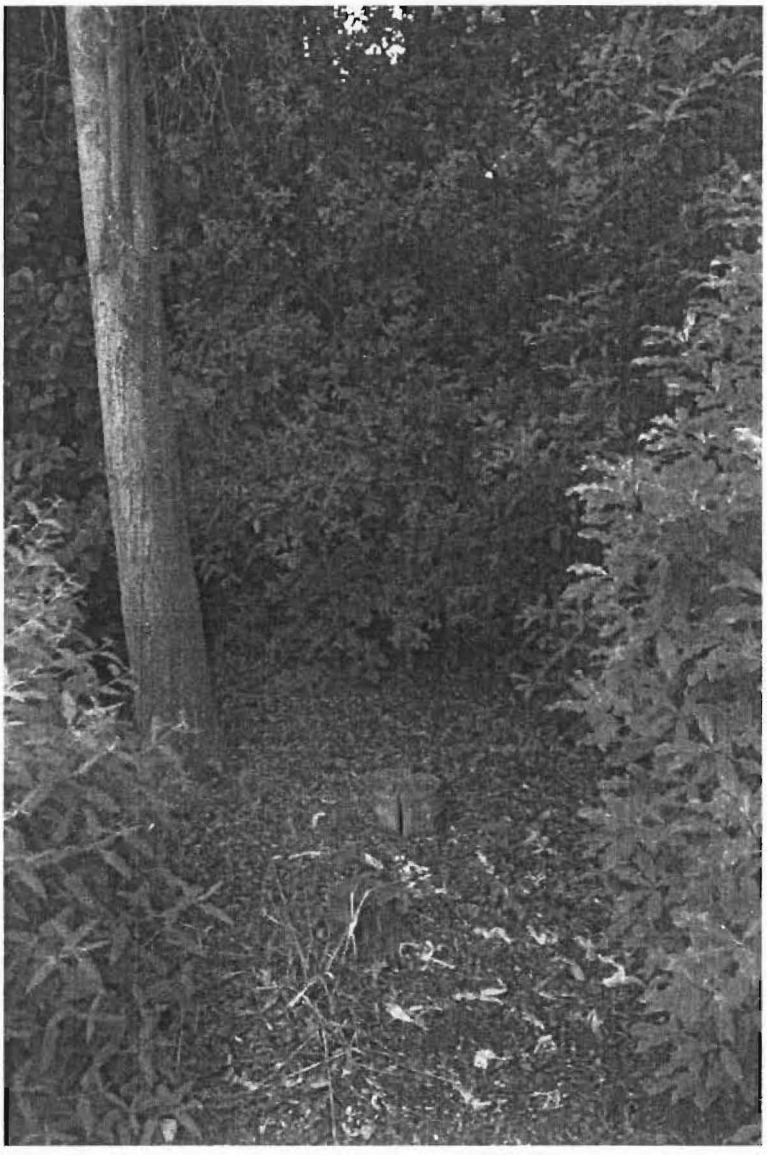
Figure 2.4 Litter bag set up.



After their respective incubation periods, the litter bags were carefully removed from the soil and air dried for approximately 14 days at room temperature (20 ± 2°C). Each bag was thoroughly cleaned of soil particles and extraneous organic material. The bags were carefully opened and the contents observed for microbial growth, invertebrate damage, and germination. The net mass loss on an oven dry, ash free basis was calculated for each litter bag. The residual contents of the litter bags were analysed for total N (Kjeldahl), moisture and ash content (refer to Section 2.3). Sub-samples of pre-incubation seeds were also analysed for moisture, ash and N content, to account for any changes in the litter quality irrespective of the soil environment.

The results of *T. aestivum* and *H. vulgare* were excluded because greater than 90% germination had occurred within 30 days, thus potentially confounding mass loss measurements.

Figure 2.5 Site of litter bag incubation in the soil



2.5.3 *In situ* decomposition of heat-treated seeds

2.5.3.1 Introduction

Live seeds confined in litter bags have the potential to germinate. Differential germination between species and replicates complicates the results of mass loss and changes in nitrogen concentration indicative of decomposition processes. To ensure no seed germination, the seeds were killed by autoclaving prior to incubation in the soil. Autoclaving is not

representative of natural conditions but is necessary to provide a universal treatment to kill all seed species.

2.5.3.2 Methodology

Autoclaved seeds (0.5-7.0 g) of *T. aestivum, H. vulgare, C. scoparius, U. europaeus, S. microphylla, P. radiata, P. contorta, N. fusca, N. solandri* and *Q. robur* were enclosed in a pre-weighed nylon mesh (14-22 μm^2) litter bags (8x5 cm). The litter bags were tagged to aid in recovery and buried at approximately 0.5 cm depth in the soil litter layer. The heat-killed litter bag experiments were carried out in the Ilam soil at the University of Canterbury campus (Figure 2.5). Three sets of litter bags were incubated *in situ* for 30, 90 or 180 days. The litter bags were set up in triplicate for each species and experimental period. Control bags were prepared as described in Section 2.5.2.2.

The litter bags were carefully removed from the soil after the assigned incubation periods. The bags were air dried for approximately 14 days at room temperature (20 ± 2°C). The litter bags were thoroughly cleaned of soil and extraneous organic matter and the content weighed. The residual seed material was analysed for moisture and ash at each incubation period. The decomposed seeds, after 180 days *in situ,* were additionally analysed for total nitrogen (Kjedahl) (refer to Section 2.3).

2.5.4 *In situ* decomposition of seeds in three different soils

2.5.4.1 Introduction

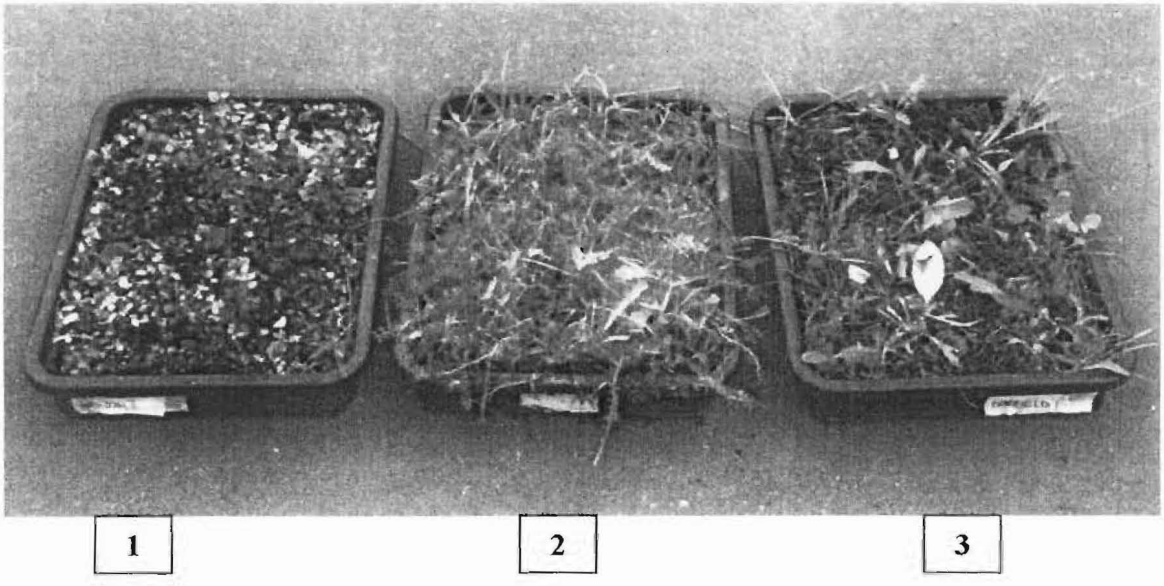
Soils vary with respect to temperature, moisture, pH, organic matter content and the quantity and quality of litter input. Soil properties determine many aspects of nutrient cycling and transformations (Howard & Howard, 1980; Vesterdal, 1999). Changes in the soil properties have the potential to alter the composition of the active microbial communities and hence the decomposition processes. Confined seeds were decomposed in 3 different soils under identical temperature and moisture conditions to investigate the influence of soil type on seed decomposition.

2.5.4.2 Methodology

Soils were collected from three sites (Site 1, 2, and 3 in Table 2.9). The pH of each soil was determined using the method outlined in Section 2.3.2. Three seed trays (35 x 50 cm) were filled with soil from the top 10 cm at each site making a total of nine seed trays.

Nylon mesh litter bags (8 x 5 cm) with 14-22 μm^2 openings were used to investigate the effect of soil type and its associated microorganisms on decomposition of *C. scoparius, U. europaeus, P. radiata, P. contorta, N. fusca* and *N. solandri*. Known quantities of whole seeds (0.4-0.5g) were sealed in the litter bags. The litter bags were randomly assigned a position in the seed trays, ensuring three replicates of each species was exposed to each soil type. The litter bags were gently pressed into the soil and covered with approximately 0.5 cm of soil. The seed trays containing soil from each site and their respective litter bags are shown in Figure 2.6.

Figure 2.6 Trays containing Hawdon soil (1), Lincoln soil (2) and Darfield soil (3).



The seed trays containing soils and litter bags were incubated in a glasshouse for 180 days. The trays were watered regularly and maintained at temperatures similar to ambient conditions. The vegetative growth in seed trays was harvested at bimonthly intervals. After 180 days, the litter bags were carefully removed and air dried at room temperature (20 ± 2°C) for 14 days. The physical state of the seeds and the microbial growth was observed and noted on a four star scale, using the schedule described in Table 2.6. The mass loss, ash and moisture were determined for each litter bag (Section 2.3.2).

2.6 STATISTICAL ANALYSIS

All statistical analysis was conducted using Statistix Version 7. One-way analysis of variances were used to determine significant differences between species substrate characteristics (Section 2.3). A correlation analysis was performed on all chemical and physical properties of the seeds (Section 2.3) to investigate linear association between pairs of variables. Each variable was tested for normality using the Shapiro-Wilk test, and transformations performed where appropriate. A correlation coefficient (r) and correlation probability function (P) was calculated for each pair of variables. The probability function tested the null hypothesis that the slope of the correlation is equal to zero. Where significant correlations were found, the correlation coefficient and its associated P value will be included in the text.

The microcosm and the *in situ* data was analysed with an analysis of variance (Sections 2.4 and 2.5). The decomposition variables were examined by the Shapiro-Wilk test to ensure the data conformed to a normal distribution. Transformations to the data were carried out where required, to normalise and homogenise the variances. The effect of treatments altering the seed substrate, the environmental conditions and the microbial communities, on the decomposition parameters were statistically analysed. A two-way analysis of variance was carried out to investigate the main effects and interactions of treatments and time on the decomposition variables. Multiple comparison tests were performed by Least Significant Difference (LSD) on all the comparative data (α=0.05). The *in situ* decomposition in different soils was analysed for each species using one-way analysis of variance.

Correlation analysis was performed on the decomposition parameters from whole, scarified, ground and heat-treated seeds and the substrate characterisation variables. Correlation coefficients and correlation probability functions were calculated for each pair of variables.

2.7 MICROSCOPIC EXAMINATION OF SEED SAMPLES

2.7.1 Introduction

Microscopy allows observations of the physical changes in the seed outer surfaces. The various treatments employed in this study have the potential to alter the physical structure of the seed coat and membrane integrity, plus provide potential entrance sites to opportunistic microflora. Microscopy observations were used to elucidate possible mechanisms or conditions promoting microbial decomposition of seed substrates.

2.7.2 Dissection microscopy

Seeds at various stages of decomposition were observed using a Wild dissection microscope. Macrophotography was carried out using a Wild Photomakrokop M400.

2.7.3 Scanning electron micrography

Scanning electron microscopy was conducted on seed samples subjected to fluctuating temperatures, wet-and-dry cycles and freeze-and-thaw cycles. The seed samples were coated with gold for 5 minutes and photographed using a Cambridge Sterocam 250 mk^2 Electron Microscope. The samples were observed within 24 hours of preparation to ensure the freshness of the samples and to avoid any artefacts from microscopy procedures.

3 RESULTS

3.1 LITTER SUBSTRATE QUALITY

3.1.1 Characterisation of seed and litter components

3.1.1.1 Seed chemical attributes

The chemical properties of the 10 surveyed seed species are presented in Table 3.1 and Table 3.2 . The values of ash, carbon, nitrogen and lipids are expressed as a percentage of oven dry mass. The measurements of seed moisture were calculated as a percentage of air dry mass. A correlation analysis was performed on all seed chemical and physical attributes to investigate the degree of association between the variables. The correlation coefficients (r) and P values are presented in the text where appropriate.

All species varied with respect to their chemical attributes. Table 3.1 describes the chemical properties of each species and their respective seed populations. The moisture content ranged between 8.4% in *P. contorta* and 14.1% in *Q. robur*. The largest lipid content was found in the *Pinus* species. Lipids comprised 32.1% and 27.8 % of the *P. radiata* and *P. contorta* mass respectively. The lowest lipid values were found in *T. aestivum* and *Q. robur*, each comprising less than 0.4% of the seed mass. Ash values ranged from 1.4% in *S. microphylla* to 7.1% in *P. radiata*.

The total nitrogen (N) concentrations varied from less than 1% in *Q. robur* to 5.84% in *U. europaeus*. The individual seeds of *Q. robur* contained the greatest absolute quantity of nitrogen (21.90 mg of nitrogen). This value greatly exceeded the other surveyed species, whose absolute N content ranged between 0.35 mg for *U. europaeus* and 2.39 mg for *S. microphylla*.

The total carbon concentration varied between 65.7% in *N. solandri* and 47.7% in *C. scoparius*. High carbon (C) content was associated with high lipid content (r=0.6932, P=0.0262). The measured values of C and N concentration were used to determine the carbon : nitrogen ratio. The carbon : nitrogen ratios (C:N) ranged between 60.8 in *Q. robur* and 11.6 in *P. contorta*. Seeds with high nitrogen concentration had low C:N ratios (r=-0.9615 P=0.0000). In most cases the C:N increased as the seed dimensions were amplified.

C:N was positively correlated with measurement of seed mass (r=0.6936, P=0.0261), length (r=0.8935, P=0.0005) and width (r=0.7885, P=0.0067).

For *U. europaeus*, *P. radiata* and *P. contorta*, two collections were made from two separate seed populations, denoted by the numeric suffixes in Table 3.1. The N concentration did vary between the seed populations of each species, however statistical analysis found these differences to be insignificant in each case. The C concentration for the multiple collections varied by approximately 3% in each species. This variation was found to be significant only in *P. radiata* (F=102.44, P=0.0096).

Table 3.1 Seed chemical attributes (% oven dry mass). Mean values of pH, moisture, ash, organic matter, lipid, N, C and C:N ratios are presented.

Species	pH	Moisture (%)	Ash (%)	Organic matter (%)	Lipids (%)	N (%)	C (%)	C : N
Triticum aestivum	5.9	13.4	1.7	98.3	0.2 ± 0.1	2.04 ± 0.09	52.6 ± 3.4	25.8
Hordeum vulgare	6.9	11.2	2.4	97.6	4.3 ± 0.00	1.19 ± 0.02	52.2 ± 1.1	43.8
Ulex europaeus 1	6.2	9.3	1.5	98.5	ND	5.62 ± 0.16	47.9 ± 0.7	8.5
Ulex europaeus 2	5.3	11.8	3.1	96.9	11.9 ± 0.0	5.84 ± 0.07	51.5 ± 0.6	8.8
Cytisus scoparius	7.2	8.7	3.6	96.4	4.3 ± 0.0	5.26 ± 0.16	47.7 ± 0.1	9.1
Pinus radiata 1	8.1	9.5	4.5	95.5	ND	4.75 ± 0.56	61.0 ± 0.2	12.8
Pinus radiata 2	4.8	9.1	7.1	92.9	32.1 ± 0.1	4.96 ± 0.47	63.0 ± 0.0	12.7
Pinus contorta 1	4.5	10.3	3.1	96.9	ND	4.26 ± 0.62	65.0 ± 0.7	15.3
Pinus contorta 2	4.8	8.4	4.7	95.3	27.8 ± 0.5	5.32 ± 0.32	61.6 ± 0.4	11.6
Nothofagus fusca	4.3	10.2	5.5	94.6	12.9 ± 0.4	2.45 ± 0.18	63.4 ± 0.2	25.9
Nothofagus solandri	4.6	12.7	1.3	98.8	18.8 ± 0.1	2.94 ± 0.08	65.7 ± 0.8	22.4
Sophora microphylla	6.6	8.5	1.4	98.6	9.9 ± 0.2	3.01 ± 0.08	56.0 ± 2.1	18.6
Quercus robur	4.8	14.1	2.8	97.2	0.4 ± 0.1	0.94 ± 0.04	57.1 ± 0.1	60.8

Note
- Errors indicated refer to the standard error of the mean. The standard errors of the means for moisture (%), ash (%) and organic matter (%) were all below 0.00 and are not included in the table.
- ND = not determined.
- The suffix 1 and 2 of *U. europaeus*, *P. radiata* and *P. contorta* denote the populations of seeds, each population collected from a separate site.

The chemical attributes of insect damaged seeds of *U. europaeus* and *S. microphylla* and the immature seeds of *U. europaeus* are shown in Table 3.2. The insect damaged seeds of both species have a reduced moisture content in comparison with the corresponding healthy seeds (Table 3.1). The insect damaged seeds of *U. europaeus* had a marginally higher C and N concentration in contrast to healthy seeds, however statistical analysis found only C to be

significantly different between the two seed populations (F=65.64, P=0.0149). The insect damaged seeds of *S. microphylla* contained comparable quantities of C to the unpredated seeds, however the nitrogen concentration was significantly lower (F=11.94, P=0.0135). As a consequence the C:N ratio was increased from 18.6 to 21.0.

The immature seeds of *U. europaeus* contained significantly less nitrogen than their corresponding mature seeds (F=23.31, P=0.0029). Mature seeds contained 5.62% N whereas immature seeds contained only 4.60% N. This relationship was reversed with respect to the C concentration. Immature seeds contained significantly higher C concentration than mature seeds (F=65.64, P=0.0149). The proportional change in C and N with seed maturity was reflected in the resultant decrease in the C:N ratio from 11.2 in immature seeds to 8.52 in mature seeds.

Table 3.2 Chemical attributes of insect-damaged seeds of *U. europaeus* and *S. microphylla* and the immature seeds of *U. europaeus* (% oven dry mass). Mean values of pH, moisture, ash, organic matter, N, C and C:N ratios are presented.

Litter type	pH	Moisture (%)	Ash content (%)	Organic matter (%)	N (%)	C (%)	C : N
U. europaeus insect damage seed	5.7	8.8	3.7	96.3	5.99 ± 0.17	56.1 ± 0.1	10.0
U. europaeus immature seed	5.8	9.4	4.1	95.9	4.83 ± 0.10	54.2 ± 0.3	11.2
S. microphylla insect damage seed	5.5	7.3	2.0	98.0	2.68 ± 0.10	56.3 ± 0.5	21.0

Note
- Errors indicated refer to standard error of the mean (SEM).
- SEM of moisture (%), ash (%) and organic material (%) were all below 0.00 and not included in the table.

3.1.1.2 Litter chemical attributes

The pH, moisture, ash, organic matter, nitrogen, carbon and the calculated carbon : nitrogen ratios of the wood and leaf litters used in the 'litter mixture' microcosm experiments (Section 3.2.9) are shown in Table 3.3. All variables are expressed on an oven dry basis, with the exception of moisture, which was calculated as a percentage of the air dry mass. The chemical properties of the leaf and wood litter fractions of *N. fusca*, *N. solandri*, *P. radiata*, and *U. europaeus* vary considerably from their corresponding seed components. The carbon concentration was reduced in all litter fractions with the exception of *U. europaeus* branch material. The carbonaceous material of *U. europaeus* comprised 57.0% and 47.9% of branch and seed material, respectively. In all wood and leaf litter components, the N

concentration was greatly reduced, ranging between 0.43% in *P. radiata* needles and 1.80% in the leguminous *U. europaeus* branches.

Table 3.3 Chemical attributes of twig and leaf litter components of *N. fusca*, *N. solandri*, *P. radiata* and *U. europaeus* (% oven dry mass). Mean values of pH, moisture, ash, organic matter, N, C, and C:N are presented.

Litter type	pH	Moisture (%)	Ash content (%)	Organic matter (%)	N (%)	C (%)	C : N
N. fusca twig	4.5	9.6	4.5	95.5	0.48 ± 0.04	48.6 ± 0.9	101.3
N. fusca foliage	4.2	9.9	7.4	92.6	1.14 ± 0.01	55.5 ± 1.2	48.7
N. solandri twig	4.5	9.3	3.9	96.1	0.66 ± 0.01	46.1 ± 0.8	69.8
N. solandri foliage	4.2	11.1	5.6	94.4	0.54 ± 0.04	49.5 ± 0.7	91.6
P. radiata needle	4.0	7.3	4.2	95.8	0.43 ± 0.00	58.1 ± 0.9	135.0
U. europaeus branch	5.5	6.4	4.1	95.9	1.80 ± 0.05	57.0 ± 0.6	31.6

Note
- Twig material of *Nothofagus* species includes only twigs less than 3mm diameter. Branch material of *U. europaeus* includes both woody and leafy material.
- The errors indicated refer to standard error of mean (SEM).
- SEM of moisture (%), ash (%), and organic material (%) were all below 0.00 and not included in the table.

3.1.1.3 Fractions of seed organic nitrogen

The distribution of organic nitrogen for each species after acid hydrolysis is shown in Table 3.4. The fractions of nitrogen are expressed as a percentage of the total seed nitrogen. The apportioning of organic N between the ammonium, α-amino acid, insoluble and hydrolysable unidentified fractions follows the same general pattern for each species, with mean values of 10.4%, 44.9%, 3.9% and 40.8% found in each component respectively. The cereal species, *T. aestivum* and *H. vulgare*, showed a comparatively larger allocation of nitrogen to ammonium-N than the other test species. The seeds of *C. scoparius* apportioned the greatest fraction of nitrogen in α-amino acids, exceeding the mean of all species by 8.4%. In all seed samples hexosamines were not present in detectable quantities.

Table 3.4 Seed organic nitrogen fractions following reflux acid hydrolysis.

Species	Ammonium-N (%)	α-amino acid-N (%)	Insoluble-N (%)	Hydrolysable Unidentified Nitrogen (%)
T. aestivum	17.6	42.6	3.3	36.5
H. vulgare	13.1	42.6	3.8	40.5
U. europaeus	9.4	44.3	2.3	44.0
C. scoparius	9.1	53.3	2.7	34.9
P. radiata	6.9	43.8	2.8	46.5
P. contorta	6.6	46.6	2.1	44.7
N. solandri	11.5	42.6	5.6	40.3
N. fusca	11.6	42.9	4.0	41.5
S. microphylla	8.3	47.2	3.9	40.5
Q. robur	10.2	43.2	8.2	38.4

Note
- Hexosamines were not present in detectable quantities.

3.1.1.4 Seed physical attributes

Each species mean seed length, width and air dry mass is displayed in Table 3.5. The mass of seeds varies considerably between species, ranging from 4.8 mg in *P. contorta* to 2329.8 mg in *Q. robur*. Generally, increases in seed mass were accompanied by increases in seed length (r=0.8891, P=0.0006) and width (r=0.8995, P=0.0004). Additionally, increases in the seed mass were associated with a reduction in seed nitrogen concentration (r=-0.6528, P=0.0407).

Water retention within and on the seed surfaces, expressed as a percentage of seed air dry mass, is also included in Table 3.5. The greatest proportional retention of water was observed in the *Nothofagus* species. Both species retained over twice their air dry mass in water. Species water retention was negatively correlated with water-soluble mass (r=-0.7699, P=0.0092).

Table 3.5 Mean seed mass, length, width and water retention.

Species	Seed mass (mg) n=100	Seed length (mm) n=50	Seed width (mm) n=50	Water retention (% air-dry mass)
T. aestivum	45.12	6.32 ± 0.09	4.24 ± 0.09	92.5
H. vulgare	40.12	9.01 ± 0.14	3.55 ± 0.08	103.3
U. europaeus 1	6.21	2.90 ± 0.06	2.17 ± 0.05	ND
U. europaeus 2	7.96	2.76 ± 0.04	2.23 ± 0.04	58.4
C. scoparius	9.60	3.54 ± 0.07	2.52 ± 0.06	46.4
P. radiata 1	31.49	7.53 ± 0.13	4.88 ± 0.31	ND
P. radiata 2	32.93	6.78 ± 0.15	4.24 ± 0.09	78.3
P. contorta 2	4.76	3.87 ± 0.10	2.15 ± 0.07	101.4
N. fusca	7.79	6.64 ± 0.12	4.57 ± 0.17	258.8
N. solandri	4.77	5.53 ± 0.14	3.56 ± 0.11	241.0
S. microphylla	79.31	6.56 ± 0.17	4.81 ± 0.09	18.4
Q. robur	2329.8	25.93 ± 0.60	14.24 ± 0.29	47.6

Note

- Errors indicated refer to the standard error of the mean.
- n = number of seeds measured.
- Suffix 1 and 2 of *U. europaeus*, *P. radiata,* and *P. contorta* denote the population of seeds, each population collected from a separate site.
- The mass, length, width, and water retention was not determined for *P. contorta* 1.

3.1.1.5 Apportioning of seed mass and nitrogen between the embryo-endosperm and the seed coat fractions

The allocation of seed mass between the seed coat and the embryo-endosperm and the nitrogen concentration of each component is presented in Table 3.6. The mass and nitrogen concentration is expressed as a percentage of oven dry mass. The allocation of mass to embryo-endosperm ranged from 92% in *H. vulgare* to 53% in *U. europaeus*. The apportioning of the seed mass between the seed coat and the embryo-endosperm shows remarkable homogeny within the *Pinus* genus. The values only diverged by 1%, despite the substantial variation in seed mass and size. The leguminous species *C. scoparius* and *U. europaeus* also showed consistencies in mass allocation, varying by only 5 %. An association between embryo-endosperm mass allocation and seed viability was present, however it was not significant (r=0.6257, P=0.0970).

The concentration of nitrogen in the embryo-endosperm varied significantly between species, ranging from 9 mg g^{-1} in *Q. robur* to 81 mg g^{-1} in *U. europaeus*. The nitrogen concentration in the seed coat varied from 4 mg g^{-1} in *H. vulgare* to 25 mg g^{-1} in *P. contorta*. The smaller seeded species generally contained higher concentration of nitrogen in the seed

embryo-endosperm fraction. The embryo-endosperm nitrogen concentration was negatively correlated with both seed length (r=-0.8348, P=0.0099) and seed width (r=-0.7510, P=0.0317). The nitrogen concentration was highest in the seed embryo-endosperm fraction, with the exception of *T. aestivum*. The seed coat of *T. aestivum* contained higher concentrations of nitrogen. However, relative to the mass of the seed coat, this was still less than the absolute quantity of nitrogen contained within the embryo-endosperm. This value of seed coat nitrogen must be interpreted with caution. The value may be inflated due to difficulty in completely separating the seed husk of *T. aestivum*.

Table 3.6 The percentage allocation of seed mass to the embryo-endosperm (EN) and seed coat (SC) fractions, the mass ratio of the two fractions and the concentration of nitrogen in the seed coat and embryo-endosperm components.

Species	EN-mass (%)	SC-mass (%)	EN:SC-mass	EN-Nitrogen ($mg\ g^{-1}$)	SC-Nitrogen ($mg\ g^{-1}$)
T. aestivum	78	22	3.5	16	21
H. vulgare	92	8	11.5	18	4
U. europaeus	53	46	1.2	81	24
C. scoparius	58	42	1.4	75	20
P. radiata	71	29	2.4	60	10
P. contorta	72	28	2.6	71	25
S. microphylla	66	34	1.9	37	9
Q. robur	72	28	2.6	9	5

Note

- EN-mass = percentage of seed mass allocated to embryo-endosperm fraction; SC-mass = percentage of seed mass allocated to seed coat fraction; EN:SC-mass = embryo-endosperm : seed coat ratio of seed mass; EN-Nitrogen = concentration of nitrogen in embryo-endosperm fraction; SC-Nitrogen = concentration of nitrogen in seed coat fraction.

3.1.1.6 Seed germination

The maximum germination potential under non-sterile conditions is presented in Table 3.7. The seed viability was expressed as the percentage of germinated seeds in the trial. The germination capacity of seeds, at the time of the trial (August 2000-January 2001), varied immensely between species. Germination rates exceeding 90% were observed in *P. radiata*, *T. aestivum* and *H. vulgare* species. In contrast, *Q. robur* seeds failed to germinate during the germination trial. Variation was also evident between the two populations of *U. europaeus* seeds, where population 2 demonstrated a two-fold germination capacity in comparison to population 1. The germination capacities in the trials were inconsistent with the germination observed in the microcosm experiments of *Q. robur* and *U. europaeus* conducted in April-May 2000. The whole seeds of *Q. robur* and the scarified seeds of *U. europaeus* exhibited

approximately 30% and 60% viability within microcosms respectively. The reductions in germination capacity between the 5 months suggest a decline in seed viability over time, under their respective storage conditions.

Table 3.7 Seed viability measured by germination trials (n=300).

Species	Viability (%)
T. aestivum	96.3
H. vulgare	99.3
U. europaeus 1	16.0
U. europaeus 2	32.7
C. scoparius	61.0
P. radiata 1	95.0
P. radiata 2	91.7
P. contorta 2	55.0
N. fusca	38.1
N. solandri	16.3
S. microphylla	40.0
Q. robur	0.0

Note
- Suffix 1 and 2 in *U. europaeus, P. radiata, P. contorta* denote the seed populations.
- Germination trials were not performed with *P. contorta* 1 population.
- n = number of seeds in germination trial for each species.

No species germinated after the trial period. In all trials microbial growth was observed after approximately 1 week, with the exception of *S. microphylla*. After seed imbibition of water, extensive fungal growth was also observed on *S. microphylla* seeds. Despite efforts to separate infected seeds, all seeds remaining in the trial were eventually colonised by fungi. The colonisation was not uniform for all individuals in the trial. The number colonised generally followed a sigmoidal curve. Only a limited number of individuals were colonised in the first week, the infection rate increased exponentially as fungi proliferated in the petri dish until all seeds were covered in microbial growth.

3.1.2 Water-soluble substances

3.1.2.1 Mass and chemical characteristics of seed water soluble extractives

Table 3.8 describes the mass and chemical characteristics of water-soluble material in each seed species, subjected to whole, scarification, heat and ground treatments. An additional seed treatment of insect damaged seeds was included for *U. europaeus* and *S. microphylla*. The mass of water-soluble material and the percentage of water-soluble nitrogen after four extractions are expressed on an oven dry basis. The concentration of ammonium-N, α-amino acid-N and reducing sugars in the leachate extracted from 1 g of seed material is also presented in Table 3.8.

Whole seeds exhibited the smallest reduction in mass after water extraction. In the case of *N. solandri*, *N. fusca* and *S. microphylla* no change in mass was detected. Scarified and heat treated seeds generally incurred more pronounced mass losses than whole seeds. However, the effect of altering seed coat integrity was not consistent for all species. The ground treatment consistently mobilised the greatest quantity of water-soluble substances (WSS) in all species. The largest mass of WSS was found in the ground leguminous *U. europaeus*, *C. scoparius* and *S. microphllya,* with a mean mass of 25.8%. The quantity of water-soluble extractives was least in the *Pinus* species with a mean value of 10.7%. A negative relationship was found between the mass of labile compounds and the seed carbon content ($r=-0.6942$, $P=0.0259$).

The proportion of water-soluble nitrogen in ground samples varied greatly between species, ranging from 39.6% in *H. vulgare* to 3.4% in *P. contorta*. Ground material released greater quantities of total nitrogen than whole, scarification and heat treatments. In the whole treatments of *U. europaeus*, *C. scoparius*, *N. solandri* and *S. microphylla* no change in nitrogen was detected. However, α-amino acid-N and ammonium-N was present in the leachate of *U. europaeus*, *C. scoparius* and *N. solandri*. The quantity of α-amino acid-N and ammonium-N found in water soluble leachates from ground samples was relatively consistent with the fractioning of nitrogen after acid hydrolysis (Table 3.4). The concentration of α-amino acid-N varied between 1024.8 $\mu g\ g^{-1}$ for *S. microphllya* and 84.0 $\mu g\ g^{-1}$ in *Q. robur* ground leachates. Ammonium-N ranged from 760.2 $\mu g\ g^{-1}$ in *N.*

fusca to 29.4 $\mu g\ g^{-1}$ in *P. contorta*. In most cases the concentration of sugar in the ground extractives exceeded the whole, scarified and heat treatments, with the exception of *N. solandri*. The ground *Q. robur* leachates exceeded all other species with 14.0 $mg\ ml^{-1}$ concentration of reducing sugars.

In the insect damaged seeds of *U. europaeus* and *S. microphylla,* the mass and nitrogen content of WSS was only quantitatively exceeded by the ground treatments. The leachate from insect damaged *U. europaeus* seeds contained the largest concentration of α-amino acid-N and reducing sugars of all seed treatments.

Table 3.9 describes the pH and microbial growth in seed leachate inoculated with soil microorganisms. In most cases, increases in pH were measured after 10 days incubation. Microbial growth was observed in all leachates within 72 hours. The extent of microbial growth is indicated on a 4 star scale, where 0 indicated the absence of microbial growth and ++++ indicated extensive bacterial turbidity and the presence of fungal flocs. Generally, microbial growth increased in the order of whole, scarified, heat and ground treatments. Incubation of the seed leachate on Plate Count Media confirmed the presence of microbial growth. The plates were dominated by moist, cream bacterial colonies. Gram stains on the dominant colonies revealed the presence of Gram negative rods in singles and pairs and 'Y'-shaped cells. Additionally, microscopic examination confirmed the presence of yeast cells. No microbial growth or change in pH was observed in the control leachates.

Table 3.8 Mass (% oven dry mass) and nitrogen (% total seed nitrogen) of the water-soluble substances released from whole, scarified, heat and ground seeds. The concentration of α-amino acid-N, ammonium-N and reducing sugars in seed leachate.

Species	Treatment	WSS mass (%)	WSS-N (%)	α-Amino acid-N ($\mu g\ g^{-1}$)	Ammonium-N ($\mu g\ g^{-1}$)	Reducing sugars ($mg\ ml^{-1}$)
T. aestivum	Whole	0.0	9.2 ± 0.02	16.8	8.4	0.1
	Scarified	0.6	2.7 ± 0.02	67.2	12.6	0.5
	Heat	0.0	6.6 ± 0.02	75.6	8.4	0.2
	Ground	13.1	23.7 ± 0.01	302.4	54.6	6.5
H. vulgare	Whole	0.6	7.8 ± 0.05	16.8	0.0	0.1
	Scarified	5.4	15.8 ± 0.03	16.8	0.0	0.2
	Heat	0.1	10.3 ± 0.01	67.2	37.8	0.2
	Ground	11.0	39.6 ± 0.03	378.0	134.4	6.3
U. europaeus	Whole	1.0	0.0 ± 0.12	117.6	42.0	0.2
	Scarified	8.2	6.5 ± 0.08	84.0	189.0	1.1
	Heat	12.0	3.2 ± 0.42	394.8	307.0	0.9
	Ground	28.8	32.5 ± 0.08	634.2	147.0	1.9
	Insect	21.8	29.4 ± 0.33	882.0	982.8	3.5
C. scoparius	Whole	1.1	22.5 ± 0.00	157.5	31.5	0.1
	Scarified	7.3	1.1 ± 0.06	336.0	142.8	1.3
	Heat	15.8	1.8 ± 0.23	147.0	168.0	0.2
	Ground	24.9	28.9 ± 0.25	697.2	151.2	2.9
P. radiata	Whole	0.0	4.4 ± 0.09	33.6	33.1	0.1
	Scarified	0.3	0.5 ± 0.03	25.2	92.4	1.1
	Heat	0.7	3.5 ± 0.05	105.0	92.4	0.2
	Ground	11.1	18.5 ± 0.15	504.0	172.2	2.5
P. contorta	Whole	1.6	3.4 ± 0.08	8.4	8.4	0.1
	Scarified	2.5	3.8 ± 0.04	58.8	8.4	1.0
	Heat	0.9	0.0 ± 0.20	8.4	0.0	0.0
	Ground	10.2	3.4 ± 0.11	197.4	29.4	0.7
N. solandri	Whole	0.0	0.0 ± 0.03	92.4	117.6	4.6
	Scarified	0.7	3.1 ± 0.08	579.6	319.2	3.3
	Heat	0.0	1.1 ± 0.04	67.2	134.4	0.1
	Ground	8.8	26.3 ± 0.01	621.6	252.0	4.2
N. fusca	Whole	0.0	9.7 ± 0.00	92.4	92.4	0.6
	Scarified	0.7	6.6 ± 0.00	75.6	92.4	0.5
	Heat	0.6	0.0 ± 0.01	306.6	630	0.9
	Ground	11.6	19.6 ± 0.06	630.0	760.2	3.3
S. microphylla	Whole	0.0	0.0 ± 0.00	8.4	0.0	0.0
	Scarified	0.0	0.0 ± 0.00	12.6	8.4	0.0
	Heat	3.1	0.0 ± 0.00	75.6	58.8	0.0
	Ground	23.8	34.6 ± 0.20	1024.8	172.2	6.6
	Insect	16.7	23.8 ± 0.06	319.2	155.4	2.7
Q. robur	Whole	2.3	ND	8.4	0.0	0.0
	Scarified	22.2	ND	46.2	29.4	3.6
	Heat	11.4	ND	0.0	75.6	3.3
	Ground	23.0	21.3 ± 0.04	84.0	63.0	14.0

- Note: WSS = Water-soluble substances; WSS-N = Water-soluble nitrogen; Insect = insect damaged seeds; ND = not determined. Errors indicated refer to standard error of mean N concentration of WSS-N.

Table 3.9 The pH of leachates from whole, scarified, heat and ground seed pre- and post-incubation with soil inoculum. Microbial growth qualified on an ordinal 4 star scale.

Species	Treatment	Initial pH	Final pH	Microbial growth
T. aestivum	Whole	6.4	8.2	+
	Scarified	6.4	7.7	++
	Heat	6.0	7.3	++++
	Ground	6.8	7.9	++++
H. vulgare	Whole	7.6	7.2	+
	Scarified	6.6	7.7	+
	Heat	6.2	7.6	+
	Ground	6.6	7.2	++++
U. europaeus	Whole	5.0	7.4	+
	Scarified	6.3	7.7	+++
	Heat	6.0	8.4	++++
	Ground	6.0	8.4	++++
	Insect	5.7	8.7	++++
C. scoparius	Whole	5.0	7.3	+
	Scarified	7.1	7.8	++
	Heat	6.0	7.5	++++
	Ground	6.3	7.9	++++
P. radiata	Whole	6.6	7.6	+
	Scarified	6.4	7.4	+
	Heat	5.2	7.0	++
	Ground	6.4	7.7	+++
P. contorta	Whole	5.6	7.2	++
	Scarified	5.1	7.6	++
	Heat	5.6	7.6	++++
	Ground	5.8	7.6	++++
N. solandri	Whole	7.2	7.4	+
	Scarified	5.5	8.0	++
	Heat	6.0	7.6	++++
	Ground	5.8	7.7	++++
N. fusca	Whole	5.4	7.4	++
	Scarified	5.4	7.3	++
	Heat	5.1	7.9	+++
	Ground	5.2	8.2	+++
S. microphylla	Whole	6.2	7.1	+
	Scarified	7.6	7.1	+
	Heat	6.5	7.6	+
	Ground	5.4	7.6	++++
	Insect	5.2	8.2	++++
Q. robur	Whole	2.7	2.3	+
	Scarified	5.0	6.5	++
	Heat	4.9	7.6	++++
	Ground	4.7	5.1	+++

Note

- Microbial growth qualitative observation range between 0-++++, where 0 represents no microbial growth and ++++ represents extensive turbidity and flocculation.

3.1.2.2 Mass of water-soluble extractives from twig and leaf litter of *N. fusca*, *N. solandri*, *U. europaeus* and *P. radiata*.

The mass of water-soluble material from ground samples of twig and leaf litter of *N. fusca*, *N. solandri*, *P. radiata* and *U. europaeus* are shown in Table 3.10. The quantity of water labile material is expressed as a percentage of initial oven dry mass. The labile extractives of *U. europaeus* represented almost 19% of branch mass, which exceeds all other species' wood and leaf litters in the study. In all cases, the mass of leaf and twig water-soluble material was much less than the corresponding seed material.

Table 3.10 Mass extracted by water leaching of ground *N. fusca*, *N. solandri*, *U. europaeus* and *P. radiata* twig and leaf litter material (% oven dry mass).

Litter type	Water-soluble substances mass (%)
N. fusca twig	4.6
N. fusca foliage	5.7
N. solandri twig	5.9
N. solandri foliage	2.8
P. radiata needle	6.9
U. europaeus branch	18.8

Note

- Twig material of *Nothofagus* species includes only twigs less than 3 mm diameter. Branch material of *U. europaeus* includes both woody and leafy material.

3.2 MICROCOSM EXPERIMENTS

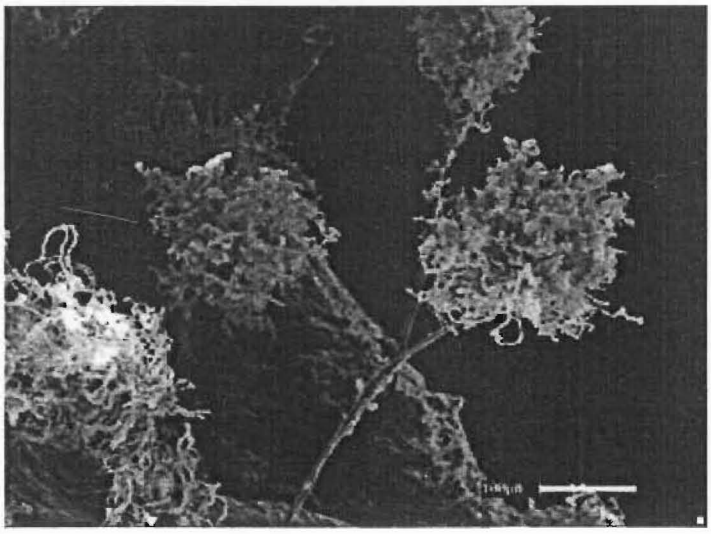
The results of all microcosm experiments are displayed in tabular or graphical form. Measurements of mass loss and net nitrogen mineralisation are expressed as a percentage of the oven dry ash free mass. References to species from this point forward will be abbreviated to two letters. The first letter of the genus and species names will denote the species. For example, *T. aestivum* and *H. vulgare* will be abbreviated to TA and HV, respectively.

3.2.1 Microbial decomposition of whole, scarified, heated and ground seeds

The mass loss and nitrogen mineralised from whole, scarified, heat and ground seeds incubated under fluctuating temperature (5-15°C) are shown in Table 3.12 and Table 3.15. All seed substrates supported extensive microbial growth, with the exception of whole *S. microphylla* (SM) seeds. Electron micrographs of *N. fusca* (NF), *C. scoparius* (CS) and *P. contorta* (PC) whole seeds, following 180 days incubation, are shown in Figure 3.1. The seed surfaces are proliferated by fungal spores and sporing structures.

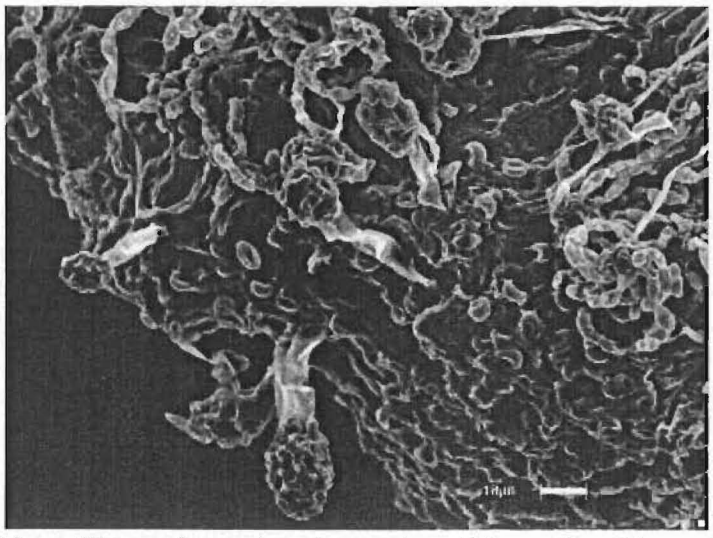
As time proceeded, the seeds became progressively discoloured and disfigured. In extreme cases, such as heat-treated *H. vulgare* (HV), *U. europaeus* (UE), and CS, the seeds progressed to an amorphous mass with individual seeds indistinguishable. After 180 days the seeds were devoid of endosperm in many cases. The volume of microbial growth increased over the first 90 day period, accompanied by increases in the microcosm pH. In the following 90 days, no significant changes in microbial growth was observed. The pattern of seed degradation was comparable in all treatments, however seed decay and discolouration proceeded at a more rapid rate in heat-treated and ground seeds. During the initial stages of decomposition, the microbial growth varied between treatments. Generally microbial growth, largely fungal, increased from whole, scarified, ground to heat-treated seeds. After 90 days incubation, there was greater variation in the quantity of microbial growth between replicates than between treatments. Germination of whole and scarified seeds within microcosms was observed for all species. The detachment of seed radicals effectively arrested seedling growth. The remains of the seedling were rapidly colonised by microorganisms.

Figure 3.1 Scanning electron micrographs of *N. fusca* (A), *C. scoparius* (B), and *P. contorta* (C) after 180 days incubation at fluctuating temperatures (5-15°C).



A

B



C

Note: The scale on the picture A = 100μm, B = 20μm, C = 10μm.

The effect of each seed treatment varied significantly between species for mass loss and nitrogen mineralisation. Table 3.11 ranks the species in descending order, according to the mean of each variable after 180 days incubation. Ranks vary according to the seed treatment and the variable measured. *T. aestivum* (TA) experienced the greatest mass loss for whole, scarified and ground treatments. The cereal species (TA and HV) incurred large mass losses in all treatments, however the species ranked consistently lower with regard to nitrogen release. UE measured the largest net nitrogen mineralisation in whole, scarified and heat treatments. In CS and SM seeds, only minimal mass loss and nitrogen release occurred from whole seeds. In treatments where the seed coat integrity was altered, scarification and heat treatments, the decomposition was considerable higher. The heat-treated seed of SM and CS measured 67% and 66% mass loss respectively following 180 days incubation. The heat-treated *Pinus* seeds experienced minimal mass losses after 180 days. However, a large fraction of the seed nitrogen was mineralised during this period.

Table 3.11 Species ranked according to mean mass loss (%) and mean net nitrogen mineralised (%) after 180 days incubation for whole, scarified, heat and ground seed treatments.

Rank	Whole		Scarified		Heat		Ground	
	Mass loss (%)	N-min (%)	Mass loss (%)	N-min (%)	Mass loss (%)	N-min (%)	Mass loss (%)	N-min (%)
1	TA (77.7)	UE (67.1)	TA (79.3)	UE (86.8)	SM (66.6)	UE (72.5)	TA (73.2)	SM (61.5)
2	HV (61.0)	PR (50.0)	HV (73.7)	PC (74.1)	CS (66.3)	PC (69.7)	SM (68.3)	UE (55.3)
3	UE (43.9)	TA (48.8)	CS (60.7)	CS (59.6)	UE (66.2)	CS (66.0)	UE (43.6)	NS (50.0)
4	NS (38.4)	PC (46.7)	UE (53.5)	NS (53.0)	TA (63.7)	PR (48.6)	NF (42.7)	TA (44.7)
5	PC (34.9)	NS (41.8)	NF (45.4)	TA (50.6)	HV (56.7)	NS (40.3)	NS (41.8)	NF (32.1)
6	PR (32.2)	HV (36.5)	NS (44.5)	PR (39.7)	NF (45.6)	SM (38.1)	PR (37.0)	PR (31.9)
7	NF (29.0)	CS (2.0)	QR (43.5)	NF (38.7)	NS (31.7)	NF (24.1)		
8	QR (25.2)	NF (0.5)	PC (33.3)	HV (32.2)	PR (20.4)	TA (21.0)		
9	CS (10.4)	SM (0.3)	SM (25.7)	SM (3.2)	PC (11.8)	HV (1.5)		
10	SM (0.7)	QR (0.1)	PR (20.4)	QR (0.0)	QR (6.9)	QR (0.0)		

Note

- Species are coded according to the first letter of the genus and species name. TA = *T. aestivum*, HV = *H. vulgare*, CS = *C. scoparius*, UE = *U. europaeus*, PR = *P. radiata*, PC = *P. contorta*, NF = *N. fusca*, NS = *N. solandri*, SM = *S. microphylla*, QR = *Q. robur*.
- Mean values of mass loss and net nitrogen mineralisation after 180 days incubation are presented in parentheses below the species code.

The mass loss and decay rate constants from whole, scarified, heat and ground treated seed are displayed in Table 3.12. Additionally, the mass loss from TA, PR, and SM are presented graphically in Figures 3.2-3.4. Table 3.13 provides a summary of the two-way analysis of variances (ANOVA) investigating the effect and interaction of seed treatment (whole, scarified, heat and ground) and time (30, 90 and 180 days) on seed mass loss.

The effect of seed treatments varied significantly between species (F=198.00, P=0.0000). In all species seed treatment and time have a significant effect on mass loss. An interaction was present in most cases indicating the two factors interact in a non-additive manner. In most species a majority of the variation in mass loss was accounted for by the incubation time. However, in CS (F=682.5, P=0.0000) and SM (F=205.7, P=0.0000) a greater part of the variation was attributed to the seed treatment.

The effect of heat treatment on seed surface morphology varied with species. The seeds of *Nothofagus* species showed an increased cellular appearance indicating the removal of surface waxes. The seeds of UE and CS showed a slight increase in the number of cracks in the seed surface. The remaining species showed extensive damage and fracturing to the seeds outer surfaces.

The decomposition curves generally conform to a linear or curvilinear pattern. In the heat-treated seeds of *Q. robur* (QR), *P. radiata* (PR), *N. solandri* (NS) and *P. contorta* (PC) and the whole seeds of PC, PR, NS and NF a lag phase was evident in the early stages of decomposition. During this period of substrate conditioning, only minimal mass loss was recorded. In most cases mass loss increased over time. However, the mass loss after 90 and 180 days were not significantly different in whole TA, QR, CS and NS seeds, characteristic of a curvilinear response. The pattern of decomposition varied significantly between treatments. Taking into account all time periods, homogeny between treatment was rarely observed. Following 180 days incubation, the difference between the treatments was less marked. After 180 days incubation no significant difference between whole and scarified seeds of PC, HV and TA was found. The heat and ground seeds of UE, NF and SM were also homogeneous at this period.

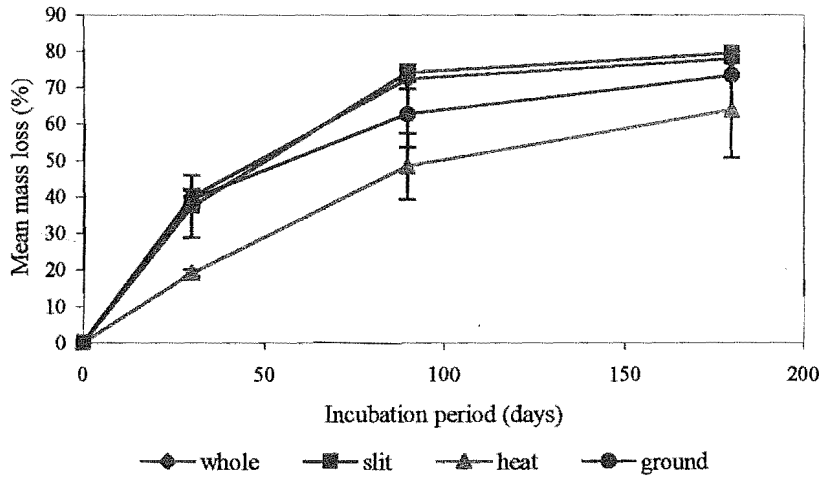
Table 3.12 Mass loss (%) at 30, 90 and 180 days from whole, scarified, heat-treated and ground seeds and the calculated decay rate constants (k).

Species	Treatment	n	Incubation period			k (0-180)
			30 d	90 d	180 d	
T. aestivum	Whole	3	40.0 ± 2.1	72.4 ± 2.8	77.7 ± 0.9	3.00
	Scarified	3	37.4 ± 8.5	74.0 ± 2.4	79.3 ± 0.54	3.15
	Heat	2	19.1 ± 1.0	48.3 ± 9.1	63.7 ± 13.0	2.03
	Ground	3	39.4 ± 2.3	62.6 ± 9.0	73.2 ± 0.4	2.63
H. vulgare	Whole	3	19.0 ± 5.6	41.5 ± 2.3	61.0 ± 5.1	1.88
	Scarified	3	29.8 ± 5.7	56.1 ± 5.3	73.7 ± 1.8	2.67
	Heat	2	7.0 ± 4.7	25.1 ± 6.4	56.70 ± 2.2	1.67
U. europaeus	Whole	3	14.1 ± 1.6	28.8 ± 1.3	43.9 ± 3.0	1.15
	Scarified	3	23.7 ± 3.5	44.4 ± 0.5	53.5 ± 1.6	1.53
	Heat	2	22.7 ± 1.3	28.6 ± 4.1	67.4 ± 0.0	2.17
	Ground	2	19.6 ± 5.1	37.0 ± 1.3	43.6 ± 1.8	1.15
C. scoparius	Whole	3	3.5 ± 0.3	7.8 ± 1.2	10.4 ± 1.7	0.22
	Scarified	3	37.4 ± 2.5	53.8 ± 0.1	60.7 ± 0.4	1.87
	Heat	2	39.8 ± 1.0	39.6 ± 4.9	66.3 ± 1.5	2.17
P. radiata	Whole	3	0.5 ± 0.2	22.4 ± 5.3	32.2 ± 4.2	0.78
	Scarified	3	3.1 ± 0.6	17.0 ± 2.0	21.8 ± 1.9	0.49
	Heat	2	0.6 ± 0.1	3.8 ± 0.6	11.8 ± 1.9	0.25
	Ground	2	16.1 ± 0.1	27.6 ± 1.9	37.0 ± 4.8	0.92
P. contorta	Whole	3	0.0 ± 0.0	18.7 ± 1.5	34.9 ± 0.6	0.86
	Scarified	3	13.3 ± 0.3	27.2 ± 0.7	33.3 ± 1.2	0.81
	Heat	2	1.8 ± 0.1	8.8 ± 1.1	20.4 ± 0.1	0.46
N. solandri	Whole	3	5.9 ± 0.2	38.8 ± 0.7	38.4 ± 2.4	0.97
	Scarified	3	15.4 ± 1.3	30.9 ± 0.4	44.5 ± 2.0	1.18
	Heat	2	1.6 ± 0.2	22.5 ± 0.4	31.6 ± 4.2	0.76
	Ground	2	10.1 ± 0.3	68.8 ± 0.9	41.8 ± 6.4	0.75
N. fusca	Whole	3	3.9 ± 0.1	12.3 ± 0.6	29.0 ± 1.6	0.68
	Scarified	3	6.9 ± 0.5	19.7 ± 1.9	45.4 ± 2.5	1.21
	Heat	2	6.9 ± 0.5	40.5 ± 0.8	45.6 ± 2.5	1.22
	Ground	2	17.6 ± 1.3	20.9 ± 2.7	42.7 ± 2.7	1.12
S. microphylla	Whole	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.00
	Scarified	3	1.3 ± 0.6	6.8 ± 1.4	25.7 ± 7.0	0.60
	Heat	3	15.1 ± 1.2	47.0 ± 1.5	66.4 ± 3.2	2.18
	Ground	3	49.9 ± 0.7	60.9 ± 0.3	68.3 ± 2.8	2.30
Q. robur	Whole	3	23.2 ± 3.4	18.2 ± 7.1	25.2 ± 3.3	0.58
	Scarified	3	18.0 ± 6.8	13.5 ± 1.5	43.5 ± 2.6	1.14
	Heat	2	0.2 ± 0.1	0.12 ± 0.0	9.7 ± 1.4	0.20

Note

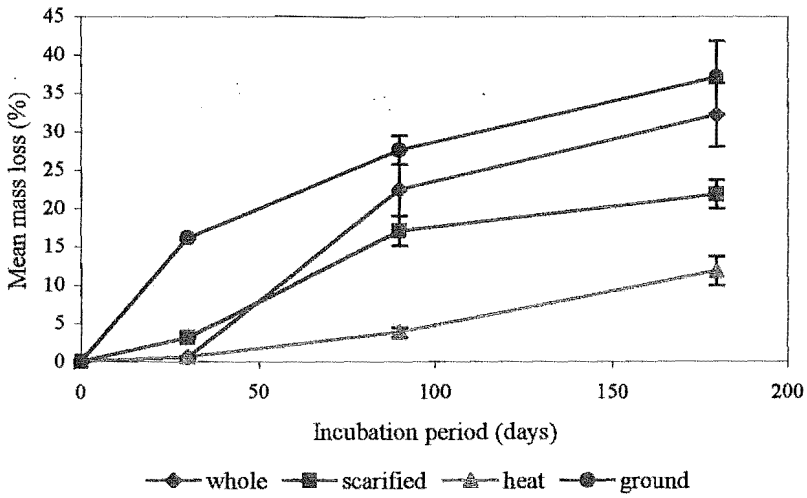
- Figures presented as mean ± standard error of the mean.
- n = number of replicates.
- Seeds were incubated at temperatures cycling between 5°C and 15°C.

Figure 3.2 Mass loss (%) from whole, scarified, ground and heat-treated seeds of *T. aestivum*.



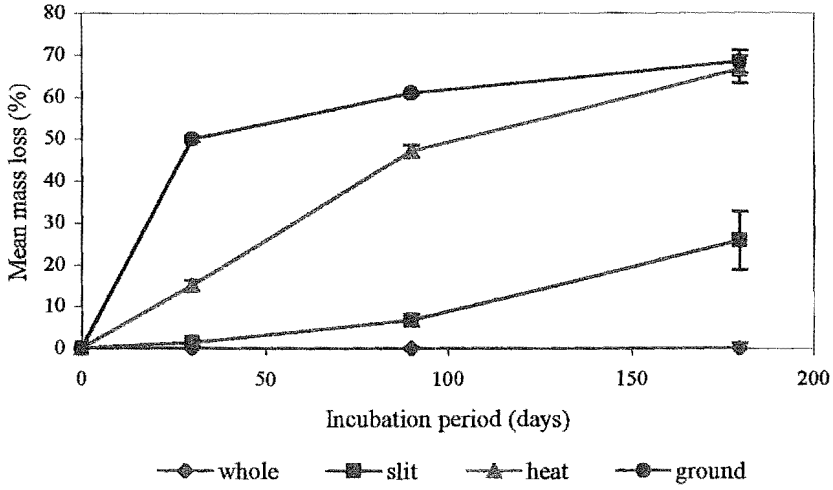
Note:
Error bars indicate the standard error of the means.

Figure 3.3 Mass loss (%) from whole, scarified, ground and heat-treated seeds of *P. radiata*.



Note:
Error bars indicate the standard error of the means.

Figure 3.4 Mass loss (%) from whole, scarified, ground and heat-treated seeds of *S. microphylla*.



Note:
Error bars indicate the standard error of the means.

Table 3.13 Summary of two-way ANOVAs testing the main and interaction effects of seed treatment (whole, scarified, heat and ground) and time (30, 90 and 180 days) on mass loss from each species.

Species	Treatment	Time	Treatment x Time
T. aestivum	17.7 ***	64.2 ***	2.8 *
H. vulgare	21.2 ***	77.6 ***	0.8 NS
P. radiata	34.8 ***	60.0 ***	3.4 *
P. contorta	223.4 ***	655.4 ***	34.7 ***
N. solandri	65.04 ***	349.38 ***	30.96 ***
N. fusca	122.5 ***	867.1 ***	42.8 ***
C. scoparius	682.5 ***	104.0 ***	22.0 ***
U. europaeus	17.6 ***	202.8 ***	10.3 ***
S. microphylla	205.7 ***	40.2 ***	9.9 ***
Q. robur	4.1 *	6.8 **	17.0 ***

Note

- The F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

The calculated decay constants (k) from each seed treatment and the mass losses measured from whole seeds at 30, 90 and 180 days were correlated with the physical and chemical characteristics of the seeds. A summary of the significant correlations is presented in Table 3.14. A correlation coefficient was calculated for all variables, and the significance of the slope analysed. The variables are arranged in descending order according to the value of the correlation coefficient.

The seed moisture content and the proportional allocation of seed mass to the embryo-endosperm fraction was positively correlated with the decay constants of whole and scarified seeds. Mass losses from heat-treated and ground seeds were negatively correlated with lipid content, nitrogen concentration and positively correlated with seed mass. Species with a high initial nitrogen concentration and embryo-endosperm : seed coat ratio displayed a reduced mass loss for heat and ground treatments.

Correlations at each incubation period of whole seeds identified potentially important variables as decomposition progressed. Throughout all time periods the mass loss was associated with proportional allocation of seed mass to the embryo-endosperm fraction and the seed moisture content. The largest mass loss, in the initial 30 days, occurred from species with low lipid, C and N content and high C:N. As time progressed, viability and water retention correlated significantly with mass loss at 90 and 180 days.

Table 3.14 Significant correlations of seed physical and chemical attributes with mass loss after 30, 90 and 180 days and decay constants (k) of whole, scarified, heat and ground seeds.

Mass loss 30 d	Mass loss 90 d	Mass loss 180 d	k whole	k scarified	k heat	k ground
MOIST (n=30) r=0.7763 P=0.0000	S-M (n=24) r=-0.6578 P=0.0005	S-M (n=24) r=-0.7242 P=0.0001	E-M (n=24) r=0.7058 P=0.0001	E-M (n=24) r=0.9204 P=0.0000	MASS (n=20) r=0.8347 P=0.0000	E-N (n=9) r=-0.9127 P=0.0006
LIP (n=30) r=-0.6582 P=0.0001	E-M (n=24) r=0.6577 P=0.0005	E-M (n=24) r=0.7233 P=0.0001	S-M (n=24) r=-0.7058 P=0.0001	S-M (n=24) r=-0.9197 P=0.0000	WID (n=20) r=0.7980 P=0.0000	LIP (n=13) r=-0.8037 P=0.0009
E-N (n=24) r=-0.6218 P=0.0012	MOIST (n=30) r=0.6270 P=0.0002	E:S (n=24) r=0.6502 P=0.0006	VIAB (n=30) r=0.5589 P=0.0013	LIP (n=30) r=-0.6761 P=0.0000	LEN (n=20) r=0.7546 P=0.0001	MASS (n=13) r=0.7079 P=0.0069
S-M (n=24) r=-0.5663 P=0.0039	E:S (n=24) r=0.4864 P=0.0159	VIAB (n=30) r=0.5355 P=0.0023	E:S (n=24) r=0.5465 P=0.0057	C (n=30) r=-0.6117 P=0.0003	C:N (n=20) r=0.5856 P=0.0067	C (n=13) r=-0.6408 P=0.0183
E-M (n=24) r=0.5657 P=0.0040	VIAB (n=30) r=0.4538 P=0.0118	MOIST (n=30) r=0.5266 P=0.0028	MOIST (n=30) r=0.5353 P=0.0023	VIAB (n=30) r=0.5322 P=0.0025	MOIST (n=20) r=0.5545 P=0.0112	E:S (n=9) r=0.6393 P=0.0063
N (n=30) r=-0.5117 P=0.0038	E-N (n=24) r=-0.4157 P=0.0433	WATRET (n=30) r=0.4502 P=0.0126	E-N (n=24) r=-0.4205 P=0.0408	E:S (n=24) r=0.4848 P=0.0163	LIP (n=20) r=-0.5207 P=0.0186	E-M (n=9) r=0.6313 P=0.0683
C:N (n=30) r=0.4882 P=0.0062	WATRET (n=30) r=0.3899 P=0.0332			MOIST (n=30) r=0.4662 P=0.0094	N (n=20) r=-0.5175 P=0.0194	N (n=13) r=-0.6064 P=0.0280
MASS (n=30) r=0.4224 P=0.0201				LAB N (n=30) r=0.4001 P=0.0285	E-N (n=16) r=-0.5166 P=0.0405	ASH (n=13) r=-0.5916 P=0.0332
C (n=30) r=-0.4165 P=0.0221					VIAB (n=20) r=-0.4688 P=0.0371	

Note

- k = decay constant, r = correlation coefficient, P = significance testing null hypothesis β=0. n = number of cases in the correlation.

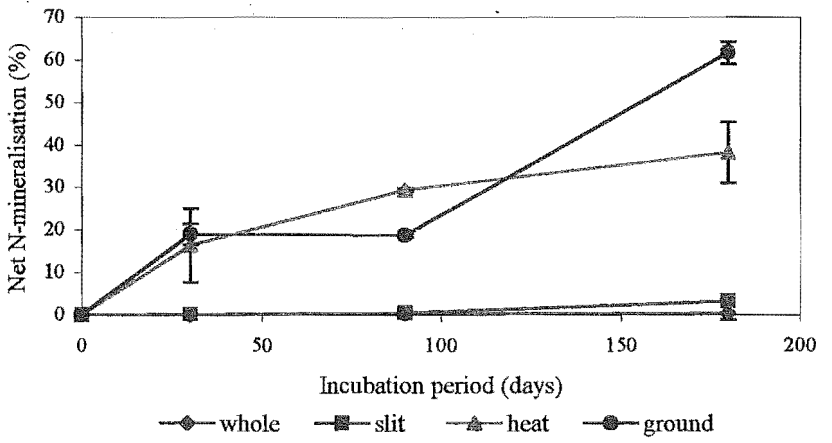
- Only significant correlations are included in the table. The correlations appear in descending order of the strength of the correlation as indicated by the size of the correlation coefficient.

- ASH = ash concentration, MOIST = moisture content, E-M = proportional allocation of mass to embryo-endosperm, E-N = nitrogen concentration in embryo-endosperm, S-M = proportional allocation of seed mass to seed coat, S-N = nitrogen concentration in seed coat, E:S = ratio of embryo-endosperm : seed coat mass, WATRET = water retention, N = nitrogen concentration, C = carbon concentration, C:N = carbon to nitrogen ratio, LIP = lipid content, LEN = seed length, WID = seed width, MASS = seed mass, VIAB = seed viability as indicated by the percentage germination, LABN = water-soluble nitrogen.

The net nitrogen release from whole, scarified, heat and ground seeds over time, expressed on an oven dry, ash free basis, is displayed in Table 3.15. A summary of the two-way ANOVAs investigating the effects of seed treatment and time on net nitrogen release is presented in Table 3.16.

No significant effect of treatment was found for QR seeds. Less than 1% mineral nitrogen was detected at all time periods and seed treatments. The whole seeds of SM, CS and NF released less than 5% nitrogen over all time periods. Alteration in the seed coat, through scarification or heat treatments, significantly increased the mineralisation of nitrogen in each species. Figure 3.5 exemplifies the enhanced nitrogen release from SM after alteration in seed coat integrity via heat treatment.

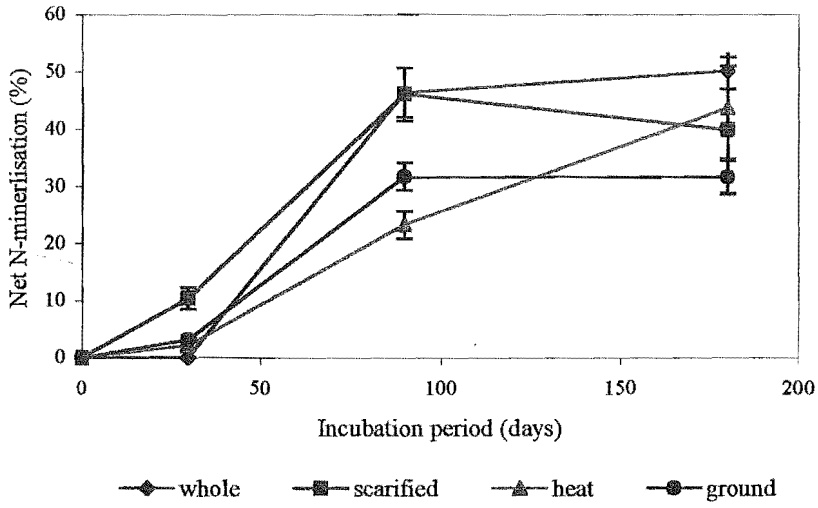
Figure 3.5 The net nitrogen mineralisation from whole, scarified, heat and ground seeds of *S. microphylla*.



Note:
Error bars indicate the standard error of the means.

The *Pinus* species displayed a similar response of net nitrogen mineralisation for all treatments. Figure 3.6 shows graphically the release of nitrogen from PR seeds. Minimal nitrogen was detected in the first 30 days. A rapid release of nitrogen occurred in the following 60 days. The rate of nitrogen release was reduced again between 90 and 180 days. Least significant difference (LSD) found no difference between nitrogen mineralised at 90 and 180 days.

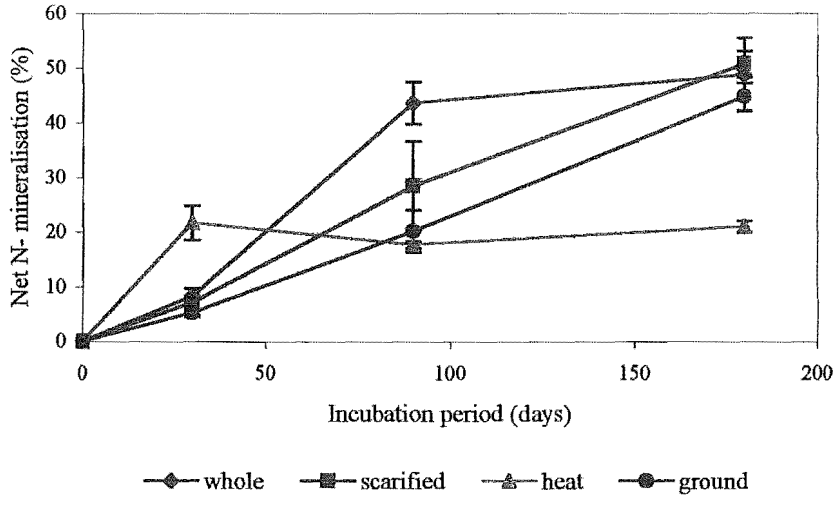
Figure 3.6 The net nitrogen mineralisation from whole, scarified, heat and ground seeds of *P. radiata*.



Note:
Error bars indicate the standard error of the means.

The nitrogen released from heat-treated seeds in TA, HV, NF, NS and CS resulted in a comparatively rapid release of nitrogen in the initial 30 days. However, after 180 days the net nitrogen release was either homogenous or less than the other treatments. This response of nitrogen release over time is shown graphically for TA in Figure 3.7.

Figure 3.7 The net nitrogen mineralisation of whole, scarified, heat and ground seeds of *T. aestivum*.



Note:
Error bars indicate the standard error of the means.

Table 3.15 Net nitrogen mineralisation (%) at 30, 90 and 180 days from whole, scarified, heat and ground treated seeds.

Species	Treatment	n	Incubation period 30 d	90 d	180 d
T. aestivum	Whole	3	8.2 ± 0.2	43.6 ± 3.9	48.8 ± 6.7
	Scarified	3	7.1 ± 2.6	28.4 ± 8.2	50.6 ± 2.4
	Heat	2	21.7 ± 3.2	17.7 ± 0.7	21.0 ± 1.1
	Ground	3	5.2 ± 0.5	20.1 ± 3.8	44.7 ± 2.5
H. vulgare	Whole	3	6.2 ± 0.6	30.9 ± 4.4	36.5 ± 9.0
	Scarified	3	2.6 ± 0.5	31.5 ± 4.5	32.2 ± 4.0
	Heat	2	7.0 ± 5.7	2.8 ± 0.0	1.5 ± 0.4
U. europaeus	Whole	3	9.9 ± 4.2	39.0 ± 2.3	69.6 ± 8.2
	Scarified	3	41.3 ± 4.0	62.9 ± 4.7	86.8 ± 1.9
	Heat	2	44.2 ± 5.2	56.1 ± 0.6	72.5 ± 0.6
	Ground	3	42.3 ± 1.7	54.4 ± 4.3	44.4 ± 1.4
C. scoparius	Whole	3	1.1 ± 0.2	3.3 ± 6.1	2.0 ± 0.1
	Scarified	3	0.1 ± 0.0	58.6 ± 6.0	59.6 ± 3.8
	Heat	2	52.0 ± 0.5	60.9 ± 0.3	66.1 ± 1.1
P. radiata	Whole	3	0.1 ± 0.0	46.3 ± 4.2	50.0 ± 3.1
	Scarified	3	10.3 ± 1.9	46.1 ± 4.6	39.7 ± 11.2
	Heat	2	2.1 ± 0.7	23.2 ± 2.4	48.6 ± 8.9
	Ground	3	3.1 ± 0.3	31.6 ± 2.4	31.5 ± 2.9
P. contorta	Whole	3	1.0 ± 0.2	32.6 ± 11.0	46.7 ± 1.0
	Scarified	3	12.0 ± 1.2	52.9 ± 9.8	74.1 ± 0.9
	Heat	2	3.7 ± 1.9	51.3 ± 2.2	69.7 ± 11.3
N. solandri	Whole	3	0.3 ± 0.0	1.2 ± 0.7	41.8 ± 4.4
	Scarified	3	12.0 ± 5.3	56.3 ± 11.0	53.0 ± 3.6
	Heat	2	29.8 ± 2.5	45.0 ± 4.6	40.3 ± 1.5
	Ground	3	15.6 ± 1.9	25.5 ± 2.7	49.3 ± 2.5
N. fusca	Whole	3	0.0 ± 0.0	0.3 ± 0.2	0.5 ± 0.2
	Scarified	3	0.38 ± 0.2	15.3 ± 4.1	38.7 ± 4.4
	Heat	2	46.0 ± 5.5	24.1 ± 5.5	24.1 ± 0.3
	Ground	3	8.95 ± 0.8	15.0 ± 2.9	42.1 ± 4.1
S. microphylla	Whole	3	0.0 ± 0.0	0.3 ± 0.2	0.3 ± 0.2
	Scarified	3	0.0 ± 0.0	0.4 ± 0.3	3.2 ± 1.5
	Heat	2	16.3 ± 8.7	29.4 ± 0.4	38.1 ± 7.2
	Ground	3	19.0 ± 2.5	18.7 ± 0.5	61.6 ± 2.7
Q. robur	Whole	3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
	Scarified	3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Heat	2	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Note
- Figures presented as mean ± standard error of the mean.
- n = number of replicates.
- Seeds were incubated at temperature cycling between 5°C and 15°C.

Table 3.16 Summary of two-way ANOVAs testing the main and interaction effects of treatment (whole, scarified, heat and ground) and time (30, 90 and 180 days) on the net nitrogen mineralisation from each species.

Species	Treatment	Time	Treatment x time
T. aestivum	6.5 **	59.1 ***	8.8 ***
H. vulgare	38.7 ***	16.0 ***	11.2 ***
P. radiata	3.6 *	78.8 ***	3.3 *
P. contorta	8.9 **	75.0 ***	0.9 NS
N. solandri	18.3 ***	43.3 ***	10.0 ***
N. fusca	96.2 ***	14.5 ***	26.2 ***
C. scoparius	195.7 ***	122.7 ***	77.4 ***
U. europaeus	29.9 ***	89.3 ***	12.7 ***
S. microphylla	74.8 ***	8.7 **	1.7 NS
Q. robur	0.5 NS	1.4 NS	3.9 *

Note: The F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

The net nitrogen mineralisation from whole seeds over time was correlated with the physical and chemical attributes of the seeds. A summary of the significant correlations, in descending order, is presented in Table 3.17. During the initial 30 days of incubation the greatest amount of nitrogen was released from seeds with lower carbon content and a reduced mass allocation to seed coat. As decomposition progressed the seeds with smaller physical dimensions and high nitrogen concentration showed the greatest net nitrogen mineralisation.

Table 3.17 Significant correlations of seed physical and chemical attributes with nitrogen mineralised after 30, 90 and 180 days.

Time						
30 days	S-M (n=24) r=-0.6247 P=0.0011	E-M (n=24) r=0.6171 P=0.0013	C (n=30) r=-0.5228 P=0.0030			
90 days	VIAB (n=30) r=0.6131 P=0.0003	WID (n=30) r=-0.3779 P=0.0395				
180 days	WID (n=30) r=-0.5128 P=0.0038	S-N (n=24) r=0.4715 P=0.0200	LEN (n=30) r=-0.4434 P=0.0141	N (n=30) r=0.4262 P=0.0188	MASS (n=30) r=-0.4142 P=0.0229	LIP (n=30) r=0.4059 P=0.0260

Note

- r = correlation coefficient, P = significance testing null hypothesis β=0. n = number of cases in the correlation.
- Only significant correlations are included in the table. The correlations appear in descending order of strength of the correlation as indicated by the size of the correlation coefficient.
- E-M = proportional allocation of mass to the embryo-endosperm, S-M = proportional allocation of mass to the seed coat, S-N = concentration of nitrogen in the seed coat fraction; N = nitrogen concentration, C = carbon concentration, LIP = lipid content; LEN = seed length, WID = seed width, MASS = seed mass, VIAB = seed viability as indicated by the percentage seed germination.

3.2.2 Decomposition of insect-damaged and immature seeds

Table 3.18 displays the resulting mass loss (%), net nitrogen mineralisation (%) and the decay constants (k) from insect-damaged seeds of UE and SM and immature seeds of UE, expressed on an oven dry, ash free basis. For comparative purposes the mass loss and nitrogen released from ground seeds is included in parentheses adjacent to the corresponding value of the insect damaged seeds. The decomposition variables of whole mature UE seeds is included in parentheses next to the immature seeds. The influences of immature and insect damaged seeds on mass loss and nitrogen release were compared with data from corresponding whole seeds. Two-way ANOVAs were performed to investigate the effects of seed type (insect damaged or immature and whole) and time (30, 90 and 180 days) on mass loss and nitrogen release. A summary of the ANOVAs is presented in Table 3.19.

Table 3.18 Mass loss, net nitrogen mineralisation and decay constants (k) from immature and insect-damaged *U. europaeus* and insect-damaged *S. microphylla* seeds (% oven dry basis) after 30, 90 and 180 days incubation.

Species	Incubation period	Mass loss (%) (n=3)	N mineralised (%) (n=3)	k (0-180)
U. europaeus immature	30	18.4 ± 0.2 (14.1 ± 1.6)	24.7 ± 4.7 (9.9 ± 4.2)	
	90	ND	35.2 ± 0.4 (39.0 ± 2.3)	
	180	44.9 ± 1.8 (43.9 ± 3.0)	63.0 ± 1.9 (69.6 ± 8.2)	1.19
U. europaeus insect-damaged	30	26.0 ± 1.0 (19.6 ± 5.1)	48.3 ± 1.8 (42.6 ± 1.7)	
	90	41.9 ± 2.1 (37.0 ± 1.3)	44.6 ± 5.9 (54.4 ± 4.3)	
	180	55.2 ± 5.7 (43.6 ± 1.8)	54.4 ± 0.6 (44.4 ± 1.4)	1.61
S. microphylla insect-damaged	30	29.1 ± 1.8 (49.9 ± 0.7)	1.6 ± 0.4 (19.0 ± 2.5)	
	90	56.6 ± 3.1 (60.9 ± 0.3)	15.0 ± 5.2 (18.7 ± 0.5)	
	180	65.2 ± 1.3 (68.3 ± 2.8)	34.4 ± 1.2 (61.6 ± 2.7)	2.11

Note
- Figures presented as mean ± standard error of the mean. ND = not determined. n = number of replicates
- Figures in parentheses in insect-damaged seeds of *U. europaeus* and *S. microphylla* refer to the mass losses and nitrogen mineralised from ground seed material. Figures in parentheses in immature seeds of *U. europaeus* refer to the mass losses and nitrogen mineralised from whole mature seed material.
- Seeds were incubated at temperatures cycling between 5°C and 15°C.

No significant difference was found in the mass loss and nitrogen released from immature and mature UE seeds. The mass loss differed only slightly by 3% after 30 days and 1% after 180 days incubation. During the initial 30 days, greater net nitrogen mineralisation was

measured from immature seeds. 9.9% nitrogen was released from mature seeds whereas 24.7% of nitrogen was released from immature seeds. This finding corresponds with the observed rapid imbibition, discolouration of seeds and fungal colonisation observed in immature UE seeds. After 180 days incubation the quantity of nitrogen mineralised from the mature seeds exceeded the immature seeds by 7%, however LSD test found the means to be homogeneous.

The insect damaged seeds of UE and SM rapidly imbibed water and exuded a coloured leachate. Following the addition of moisture, the seeds were colonised by fungi within 72 hours. Electron micrographs in Figure 3.8 show the intimate association of fungal hyphae with the insect damaged sites of the seed coat of SM. A small number of the insect damaged seeds of SM and the immature seeds of UE retained the ability to germinate. The emerging radicals were extensively colonised by microbes.

Table 3.19 Summary of two-way ANOVAs testing the main and interaction effects of seed condition and time on the mass loss and net nitrogen mineralisation from *S. microphylla* and *U. europaeus*.

	Mass loss			N-mineralised		
Species	Seed condition	Time	Seed condition x Time	Seed condition	Time	Seed condition x Time
U. europaeus immature	0.15 NS	45.32 ***	1.42 NS	0.26 NS	90.69 ***	5.07 *
U. europaeus insect-damaged	31.58 ***	76.68 ***	0.08 NS	9.12 *	36.51 ***	24.17 ***
S. microphylla insect-damaged	1835.88 ***	43.18 **	28.55 **	102.49 ***	8.21 **	2.05 NS

Note

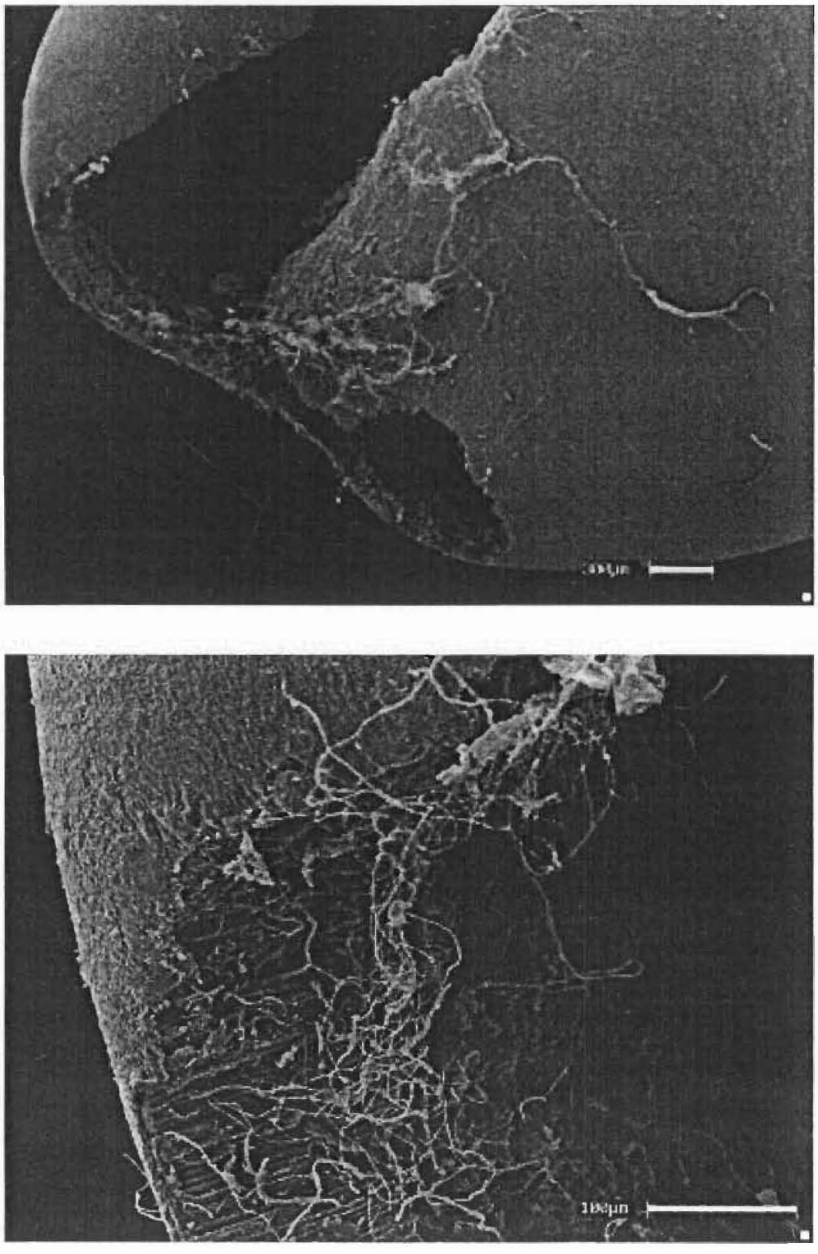
- F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

In the UE and SM seeds, insect damaged seeds surpassed the mass loss and net nitrogen mineralisation from the corresponding whole seeds, at all incubation periods. The effect of seed condition on SM seeds accounted for the greatest portion of variation in the mass loss (F=1835.88, P=0.0000) and nitrogen release (F=102.49, P=0.0000). The 65.5% mass loss and 34.4% nitrogen release after 180 days incubation, of insect-damaged SM seeds, greatly exceeded minimal mass loss and mineral nitrogen measure from whole seeds.

The mass loss from insect damaged UE seeds exceeded whole UE seeds by approximately 10% at each time period. The release of nitrogen after 30 days was 40% greater in insect-

damaged seeds. As decomposition proceeded, the difference between the two conditions was reduced with 69.6% and 54.4% of the seed nitrogen mineralised after 180 days from insect-damaged and whole seeds respectively.

Figure 3.8 Electron micrographs of the insect-damaged seeds and the associated fungal growth of *S. microphylla*, prior to incubation.



A

B

Note: The scale in picture A = 300μm, B = 100μm.

3.2.3 Microbial decomposition under a constant temperature regime

The mean mass loss, net nitrogen mineralisation, and decay constants under a constant temperature regime of 20°C are shown in Table 3.20. The mass loss and nitrogen release was expressed on an oven dry, ash free basis. Mass losses increased over time in all species. In the TA and HV seeds, mass losses after 90 and 180 days incubation were not significantly different. In most species net nitrogen mineralisation increased over time, with the exception of the *Nothofagus* species. NF and NS seeds underwent an initial period of net nitrogen mineralisation followed by nitrogen immobilisation.

The effect of constant (20°C) and fluctuating (5-15°C) temperature regimes on the microbial decomposition, over time, were contrasted using a two-way ANOVA. The main and interaction effects of temperature (constant and fluctuating) and time (30, 90 and 180 days) were analysed for significant effects on mass loss and nitrogen mineralisation. A summary of the two-way ANOVAs is presented in Table 3.21.

A significant effect of temperature on mass loss was found in all species with the exception of UE and SM. Generally, mass losses were greater under a constant temperature regime. LSD of the means found the mass losses incurred after 90 days incubation under constant temperatures were statistically similar to mass losses after 180 days incubation under fluctuating temperatures in CS, HV, NS, NF and PR seeds. The significant interaction of temperature and time on TA, PC, NS and UE indicates the effect of temperature was dependent on the time course of decomposition.

The effect of temperature on nitrogen release was only significant in PR, NS, NF and UE seeds. The mean release of mineral nitrogen over all incubation periods was greater under constant temperatures for all species with the exception of TA and UE. The N released from UE seeds under fluctuating conditions exceeded constant conditions by 16%. The PR seeds incubated under a constant temperature released 11% more N after 180 days than fluctuating temperatures.

Table 3.20 The mass loss, net nitrogen mineralisation and decay rate constants (k) of whole seeds incubated under a constant temperature regime (20°C).

Species	Incubation period (days)	Mass loss (%) (n=2)	N mineralised (%) (n=2)	Mass loss k (0-180 d)
T. aestivum	30	63.4 ± 0.1	8.4 ± 3.4	
	90	74.8 ± 0.4	44.2 ± 4.3	
	180	76.8 ± 2.3	41.2 ± 2.6	2.92
H. vulgare	30	41.8 ± 4.0	8.4 ± 1.1	
	90	71.6 ± 2.8	23.3 ± 7.9	
	180	77.6 ± 0.7	29.9 ± 12.9	2.99
P. radiata	30	23.2 ± 3.9	4.2 ± 0.0	
	90	27.2 ± 9.1	49.7 ± 3.9	
	180	45.8 ± 4.0	61.0 ± 3.2	1.23
P. contorta	30	20.4 ± 2.0	19.1 ± 3.3	
	90	19.7 ± 0.8	36.8 ± 2.1	
	180	38.2 ± 1.5	35.3 ± 18.8	0.96
N. solandri	30	13.1 ± 1.6	8.3 ± 2.5	
	90	37.1 ± 0.4	17.1 ± 4.5	
	180	47.7 ± 3.4	4.2 ± 1.9	1.29
N. fusca	30	25.8 ± 7.1	41.7 ± 14.1	
	90	38.4 ± 0.2	5.8 ± 1.7	
	180	46.6 ± 0.7	2.9 ± 0.6	1.25
C. scoparius	30	7.6 ± 0.7	1.5 ± 0.5	
	90	8.8 ± 0.4	2.8 ± 1.1	
	180	15.2 ± 0.5	7.2 ± 2.5	0.33
U. europaeus	30	12.9 ± 0.1	12.2 ± 2.2	
	90	20.3 ± 1.0	17.2 ± 2.6	
	180	51.6 ± 1.9	54.3 ± 2.6	1.45
S. microphylla	30	0.0 ± 0.5	0.3 ± 0.2	
	90	0.0 ± 0.3	0.2 ± 0.0	
	180	0.0 ± 0.2	0.3 ± 0.2	0.00
Q. robur	30	1.7 ± 1.1	0.0 ± 0.0	
	90	10.8 ± 6.0	0.0 ± 0.0	
	180	17.5 ± 6.5	0.0 ± 0.0	0.38

Note
- Figures presented as mean ± standard error of the mean.
- n = number of replicates.

Table 3.21 Summary of two-way ANOVAs testing the main and interaction effects of temperature (constant and fluctuating) and time (30, 90 and 180 days) on the mass loss and net nitrogen mineralisation.

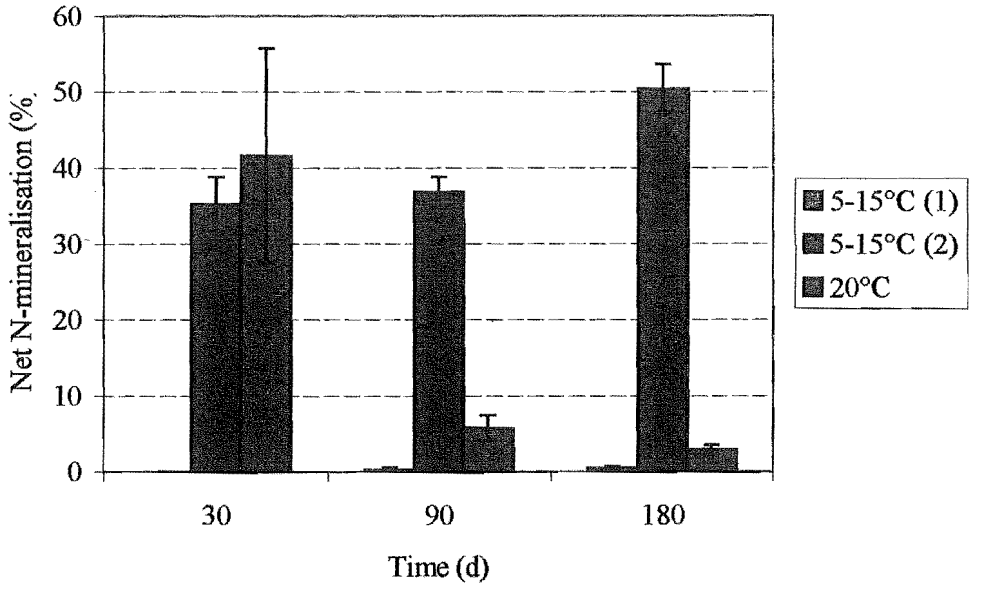
	Mass loss			Nmin		
Species	Temp	Time	Temp x Time	Temp	Time	Temp x Time
T. aestivum	31.63 ***	116.06 ***	26.35 ***	0.66 NS	47.10 ***	1.13 NS
H. vulgare	51.22 ***	50.04 ***	1.43 NS	0.09 NS	11.54 **	0.42 NS
P. radiata	14.12 **	19.82 **	2.15 NS	7.28 *	210.52 ***	1.09 NS
P. contorta	83.67 ***	334.85 ***	52.59 ***	0.36 NS	9.84 *	1.99 NS
N. solandri	12.28 **	262.15 ***	6.91 *	25.62 ***	20.98 ***	43.03 ***
N. fusca	162.10 ***	59.80 ***	2.04 NS	85.69 ***	0.93 NS	8.73 *
C. scoparius	15.28 **	26.24 ***	2.10 NS	0.30 NS	4.41 *	3.83 NS
U. europaeus	1.49 NS	186.15 ***	9.78 **	15.75 **	105.08 ***	6.14 *
Q. robur	14.18 **	1.74 NS	1.20 NS	0.00 NS	7.69 *	0.07 NS
S. microphylla	2.06 NS	0.36 NS	0.32 NS	0.08 NS	0.47 NS	0.48 NS

Note

- F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

The F values of the mass loss and the N mineralised from NF seeds showed a majority of the variation was associated with the temperature regime (Table 3.21). Greater mass loss and nitrogen was released from seeds under a constant temperature. The experiments under constant temperature were initiated four months after the experiments under fluctuating conditions. As a consequence, the seeds decomposed under a constant temperature regime were older, which had the potential to confound any comparative results. To investigate any confounding effects, the decomposition of NF seeds under a fluctuating temperature regime were repeated using the older NF seeds. The second trial measured a 35.2%, 36.8% and 50.4% nitrogen release at 30, 90 and 180 days respectively. These figures greatly exceeded the values obtained in the first trial where only 3.1% of the nitrogen was released after 180 days. The nitrogen released from first and second trial under fluctuating temperatures and the nitrogen released under constant temperatures are presented in Figure 3.9. The two-way ANOVA investigating the effects of temperature and time was repeated using the results from the second trial. A significant difference was found between temperature regimes (F=22.24, P=0.0033), with nitrogen mineralisation under fluctuating conditions exceeding constant conditions.

Figure 3.9 Net nitrogen mineralised from *N. fusca* seeds under fluctuating and constant temperatures



Note
- The numerical values in parentheses refer to the first (1) and second (2) trial of seed decomposed under fluctuating temperatures (5-15°C).
- Error bars indicate standard error of the means.

3.2.4 Decomposition following the removal of labile extractives

The net nitrogen mineralisation following the removal of water-soluble material from whole seeds, incubated at temperature fluctuating between 5-15°C, is displayed in Table 3.22. Measurements of the net nitrogen mineralisation from each species after 30 and 90 days were compared with the nitrogen mineralised from whole seeds not subjected to extraction processes (control). One-way ANOVA on the data pooled from all species found significantly more nitrogen was released from labile-free seeds in the first 30 days ($F=7.75$, $P=0.0078$). The mean of all test species varied from 2.4% in control seeds to 9.5% in labile-free seeds. After 90 days incubation there was no significant difference between labile-free and control seeds ($F=2.13$, $P=0.1510$), with mean values of 28.8% and 19.8% respectively.

Table 3.22 Mean nitrogen mineralised from seeds after 30 and 90 days decomposition following the removal of labile compounds.

	Incubation period	
Species	30 d (n=2)	90 d (n=2)
T. aestivum	14.3 ± 5.3	50.1 ± 2.7
H. vulgare	8.3 ± 3.0	31.3 ± 5.9
P. radiata	0.4 ± 0.2	51.5 ± 1.8
P. contorta	2.2 ± 0.2	30.3 ± 2.5
N. solandri	34.0 ± 0.8	58.3 ± 8.6
N. fusca	29.7 ± 3.6	46.8 ± 11.3
C. scoparius	1.3 ± 0.2	4.5 ± 0.6
U. europaeus	4.3 ± 0.8	14.9 ± 1.6
Q. robur	0.0 ± 0.0	0.1 ± 0.0
S. microphylla	0.2 ± 0.0	0.3 ± 0.0

Note

- Figures presented as mean ± standard error of the mean.
- n = number of replicates.
- Seeds were incubated at temperatures cycling between 5°C and 15°C.

Two-way ANOVAs were performed on each individual species to investigate the effect of seed treatment (labile-free and control) and time (30 and 90 days). Time was significant in all species with the exception of SM, NF and NS. Only negligible amounts of mineral nitrogen were detected at each incubation period from SM. Significant effects of seed treatment were found in NS (F=149.51, P=0.0000), NF (F=98.24, P=0.0006), UE (F=27.88, P=0.0019) and PR (F=12.90, P=0.0115). In PR, NS and NF seeds, significantly more nitrogen was mineralised from labile-free seeds at both time periods. In UE the initial net nitrogen mineralised after 30 days was homogeneous between seed treatments. After 90 days incubation, 24% more nitrogen was released from the control seeds. The results of NF seeds were contrasted with a second control containing seeds of an equivalent age, for the reason discussed in Section 3.2.3. The two-way ANOVA found no significant effect of seed treatment (F=0.13, P=0.7388) or time (F=2.25, P=0.2084).

3.2.5 Decomposition of seeds with native soil inoculum

Table 3.23 displays the mass loss, the net nitrogen mineralised and the decay constants of whole PR, NS and CS seeds inoculated with Darfield, Hawdon, and Lincoln soil inoculums respectively. The decomposition variables were expressed on an oven dry, ash free basis. The inoculations were collectively referred to as native soil inoculums in the present study. The results from seeds incubated with native soil microorganisms were compared with seeds inoculated with Ilam soil microorganisms. A two-way ANOVA was performed testing the main and interaction effects of the soil inoculum (native and Ilam) and time (30, 90, and 180 days). A summary of the ANOVAs is displayed in Table 3.24.

Table 3.23 Mass loss, net nitrogen mineralisation and decay rate constants (k) of whole seeds inoculated with native soil microorganisms after 30, 90 and 180 days.

Species	Incubation period	Mass loss (%) (n=2)	N mineralised (%) (n=3)	Mass loss k (0-180 d)
P. radiata	30	0.4 ± 0.1	0.8 ± 0.4	
	90	22.9 ± 2.2	51.0 ± 0.6	
	180	42.2 ± 1.3	66.2 ± 4.6	1.10
N. solandri	30	4.9 ± 0.9	20.4 ± 0.4	
	90	32.9 ± 0.3	49.3 ± 5.4	
	180	41.4 ± 0.8	53.6 ± 4.4	1.07
C. scoparius	30	2.7 ± 0.1	3.3 ± 0.2	
	90	6.2 ± 2.2	8.8 ± 1.5	
	180	11.5 ± 0.6	8.6 ± 0.9	0.24

Note
- Figures presented as mean ± standard error of the mean.
- n = number of replicates.
- Seeds were incubated at temperatures cycling between 5°C and 15°C.

The effect of soil inoculum on mass loss was not significant in any of the species tested. All species inoculated with native soil microorganisms released significantly more nitrogen at each incubation period. LSD found the nitrogen mineralised at 90 and 180 days were homogenous in each species.

The conclusions drawn from this experiment must be done with caution. The experiments of seeds with native soil inoculum were carried out 5 months after seed decomposition experiments using Ilam soil inoculum. The seeds physical, chemical and physiological

conditions may have varied between time periods, potentially confounding any comparative results.

Table 3.24 Summary of two-way ANOVAs testing the main and interaction effects of soil inoculum (native and Ilam microorganisms) and time (30, 90 and 180 days) on the mass loss and net nitrogen mineralisation of *P. radiata*, *N. solandri* and *C. scoparius*.

	Mass loss			N mineralised		
Species	Soil inoculum	Time	Soil inoculum x Time	Soil inoculum	Time	Soil inoculum x Time
P. radiata	1.89 NS	72.13 ***	1.73 NS	9.65 **	83.32 ***	3.81 NS
N. solandri	1.73 NS	479.95 ***	6.67 *	94.01 ***	62.28 ***	15.81 ***
C. scoparius	0.18 NS	25.49 ***	0.79 NS	38.75 ***	8.52 **	3.40 NS

Note
- F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

3.2.6 Decomposition of seeds in the presence of endophytes

The net nitrogen mineralisation (% oven dry basis) of PR and PC seeds, in the absence of soil inoculum is shown in Table 3.25. Two-way ANOVAs tested the effects of soil inoculum (presence and absence) and time (30, 90 and 180 days) on the net mineralisation of nitrogen. A significant effect of soil inoculum was only found in PR seeds (F=7.85, P=0.0160). The nitrogen mineralised in the presence and absence of soil microorganisms was homogenous at 30 and 90 days incubation, varying by less than 1%. However, the nitrogen released after 180 days increased by 10% in the absence of soil microorganisms.

Table 3.25 Net nitrogen mineralised (%) from *P. radiata* and *P. contorta* seeds in the absence of soil microorganisms after 30, 90 and 180 days incubation.

Species	Incubation period	N mineralised (%) (n=3)
P. radiata	30	0.1 ± 0.0
	90	50.7 ± 0.7
	180	60.9 ± 0.8
P. contorta	30	0.8 ± 0.2
	90	23.9 ± 1.4
	180	48.2 ± 3.3

Note
- Errors indicated refer to standard errors on the mean.
- n = number of replicates.

3.2.7 Decomposition of seeds subjected to freeze-and-thaw cycles

The mean percentage mass loss, the net nitrogen mineralisation and the decay constants of whole seeds subjected to repetitive freeze-and-thaw cycles are shown in Table 3.26. The decomposition parameters are expressed on an oven dry, ash free basis. Table 3.27 presents a summary of the two-way ANOVAs testing the effects and interactions of temperature regime (constant and freeze-and-thaw) and time (30, 90 and 180 days) on mass loss and net nitrogen mineralisation.

The mass loss after 180 days incubation, under cyclical freeze-and-thaw conditions, vary from 0.4% in SM to 19.6% in UE seeds. In all cases the resulting mass losses were significantly less than those incurred under a constant temperature regime. The nitrogen released from UE, NS, NF and CS seeds subjected to freeze-and-thaw cycles, was comparable to, or exceeded, the net nitrogen mineralisation under a constant temperature regime. LSD found the net nitrogen mineralisation after 180 days under constant and freeze-and-thaw conditions were not significantly different in UE and CS species, varying only 1% and 6% respectively. *Nothofagus* species mobilised significantly more nitrogen under freeze-and-thaw conditions in contrast to seeds maintained at a constant temperature. 180 days of repetitive freeze-and-thaw temperatures enhanced the release of nitrogen by 54.5% and 48.2% in NS and NF seeds respectively. The *Pinus* species mineralised less nitrogen under freeze-and-thaw conditions. The quantity of mineral nitrogen after 180 days was found by LSD to be statistically similar to mineral nitrogen present after 30 days under constant temperatures.

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Table 3.26 Mass loss (%), net nitrogen mineralisation (%) and decay rate constants (k) from seeds subjected to repetitive freeze-and-thaw cycles for 30, 90 and 180 days.

Species	Incubation period (days)	Mass loss (%) (n=2)	N mineralised (%) (n=2)	Mass loss k (0-180d)
P. radiata	30	3.6 ± 0.1	3.7 ± 0.2	
	90	8.1 ± 2.2	2.5 ± 1.0	
	180	19.4 ± 3.3	0.5 ± 0.2	0.43
P. contorta	30	2.1 ± 0.2	2.2 ± 0.2	
	90	5.0 ± 0.1	7.1 ± 0.2	
	180	13.5 ± 0.9	13.0 ± 1.9	0.29
N. solandri	30	5.0 ± 0.3	8.0 ± 0.8	
	90	11.6 ± 3.0	46.5 ± 2.6	
	180	18.1 ± 0.0	59.3 ± 1.2	0.40
N. fusca	30	1.6 ± 0.0	15.6 ± 1.7	
	90	5.2 ± 1.0	30.8 ± 1.8	
	180	10.9 ± 0.3	51.2 ± 0.6	0.23
C. scoparius	30	0.9 ± 0.2	1.7 ± 0.3	
	90	6.8 ± 0.4	4.7 ± 0.8	
	180	14.6 ± 3.4	13.8 ± 1.6	0.32
U. europaeus	30	9.3 ± 0.1	10.1 ± 0.8	
	90	11.3 ± 0.2	45.3 ± 3.8	
	180	19.6 ± 6.0	53.7 ± 3.9	0.44
S. microphylla	30	0.0 ± 0.7	0.3 ± 0.0	
	90	0.0 ± 0.4	0.2 ± 0.0	
	180	0.4 ± 0.2	0.3 ± 0.1	0.01

Note
- Figures presented as mean ± standard error of the mean.
- n = number of replicates.

Table 3.27 Summary of two-way ANOVAs testing the main and interaction effects of temperature (constant and freeze-and-thaw) and time (30, 90 and 180 days) on mass loss and net nitrogen mineralisation of each species.

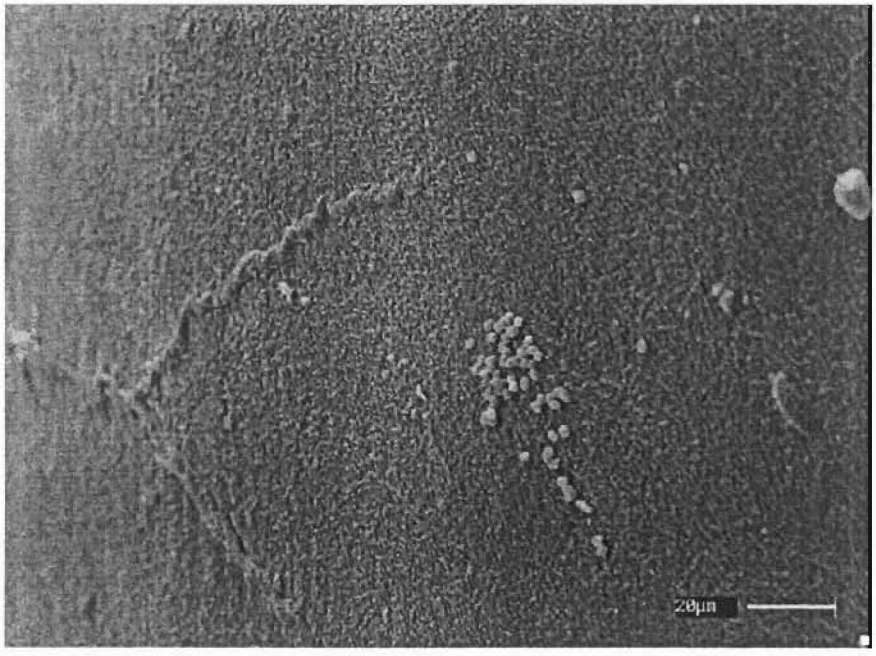
	Mass loss			N-mineralised		
Species	Temperature	Time	Temperature x Time	Temperature	Time	Temperature x Time
P. radiata	31.95 **	9.16 *	0.41 NS	108.99 ***	4.99 NS	28.56 **
P. contorta	379.01 ***	95.02 ***	13.16 **	12.84 *	1.68 NS	0.33 NS
N. solandri	130.18 ***	58.33 ***	16.09 **	37.91 ***	12.92 **	15.43 **
N. fusca	166.47 ***	13.10 **	2.13 NS	10.33 *	1.76 NS	20.42 **
C. scoparius	6.54 *	28.41 ***	2.51 NS	3.54 NS	19.69 **	0.36 NS
U. europaeus	22.30 **	31.41 ***	5.97 *	7.29 *	90.88 ***	13.84 **
S. microphylla	2.87 NS	3.72 NS	5.42 NS	0.02 NS	0.38 NS	0.15 NS

Note: F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

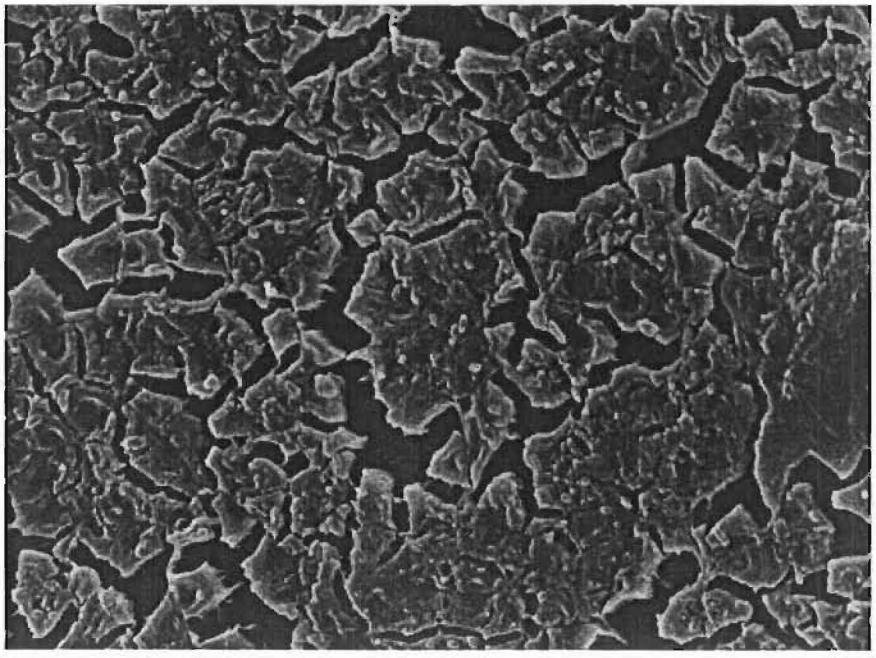
No imbibition or microbial growth was evident in SM seeds over all incubation periods. Electron micrographs showed the increased number of micro-cracks (<2μm wide) on the seed coat of seeds subjected to freeze-and-thaw conditions and the presence of bacillus-shaped bacteria on the seed surface. The other test species supported only minimal microbial growth. The presence of microbial populations on all seeds was confirmed in scanning electron micrographs. The seeds of most species imbibed rapidly and became soft within 90 days of repetitive freeze-and-thaw cycles. The seed coat of CS showed extensive cracks in comparison to seeds maintained at 5-15°C (Figure 3.10).

The seeds of most species were surrounded by a turbid or coloured leachate. A pungent odour was present in all microcosms. After 180 days the *Pinus* seeds exuded a cream liquid from lateral sides when slight pressure was applied. During the later stages of decomposition the seeds of UE and CS appeared flat and devoid of endosperm. No seed germination occurred for any species under freeze-and-thaw conditions.

Figure 3.10 Scanning electron micrographs of *C. scoparius* seeds before (A) and after (B) subjection to repetitive freeze-and-thaw cycles for 180 days (magnification x 2000).



A



B

Note: Scale = 20µm.

3.2.8 Decomposition of seeds subjected to wet-and-dry cycles

The mean mass loss, net nitrogen mineralisation and decay constants from seeds subjected to repetitive wet-and-dry cycles are shown in Table 3.28. All values are expressed on an oven dry, ash free basis. Table 3.29 presents a summary of the two-way ANOVAs testing the effect and interaction of moisture regime (constant and wet-and-dry) and time (30, 90 and 180 days) on the decomposition parameters.

Table 3.28 Mass loss (%), net nitrogen mineralisation (%) and decay constants (k) of seeds subjected to repetitive wet-and-dry cycles for 30, 90 and 180 days.

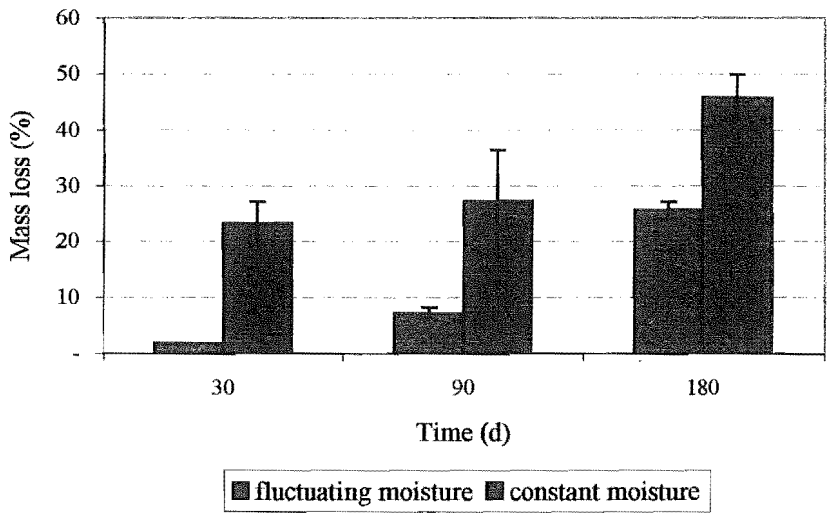
Species	Incubation period (d)	Mass loss (%) n=2	Net N mineralised (%) n=2	Mass loss k (0-180 days)
P. radiata	30	1.8 ± 0.1	5.9 ± 1.1	
	90	7.1 ± 1.0	9.5 ± 1.4	
	180	25.6 ± 1.6	6.1 ± 1.8	0.59
P. contorta	30	6.2 ± 5.0	1.2 ± 0.2	
	90	7.4 ± 0.4	9.2 ± 0.5	
	180	15.4 ± 1.2	8.7 ± 0.6	0.33
N. solandri	30	22.9 ± 3.8	0.1 ± 0.3	
	90	21.3 ± 0.1	14.4 ± 5.1	
	180	39.9 ± 3.1	10.3 ± 1.5	1.02
N. fusca	30	3.9 ± 0.7	5.9 ± 1.1	
	90	19.8 ± 3.6	9.5 ± 1.4	
	180	35.2 ± 3.2	6.1 ± 1.8	0.87
C. scoparius	30	2.0 ± 1.4	1.1 ± 0.7	
	90	9.1 ± 1.5	1.7 ± 0.4	
	180	11.0 ± 6.0	2.5 ± 1.4	0.23
U. europaeus	30	10.6 ± 3.1	1.9 ± 0.4	
	90	19.7 ± 4.6	5.7 ± 1.0	
	180	38.5 ± 3.3	12.7 ± 2.2	0.97
S. microphylla	30	0.0 ± 0.5	0.0 ± 0.0	
	90	0.0 ± 0.2	0.4 ± 0.0	
	180	0.4 ± 0.1	0.6 ± 0.3	0.01

Note
- Figures presented as mean ± standard error of the mean.
- n = number of replicates.

Mass loss from seeds exposed to repetitive wetting-and-drying conditions varied from 0.4% in SM to 39.9% in NS. All species incurred greater mass losses under conditions of constant moisture. However, this was only found to be significant in PR, NF, SM and PC seeds. The greatest effect of intermittent moisture was observed in the *Pinus* species. The period

required for 95% turnover of seed mass was extended from 3.1 years to 9.0 years in PC and 2.4 years to 5.1 years in PR. Where significant effects of the moisture regime were present, the greatest differences were found in the early stages of decomposition. The intermittent moisture regime provided seeds with optimum conditions for decomposition for approximately half the time of the corresponding seeds maintained continuously under optimum moisture. This would suggest that the mean mass loss after 90 days incubation under constant moisture should be similar to 180 days with fluctuating moisture. LSD of CS, PR and PC means supported this hypothesis. The mass losses after 90 days under a constant moisture regime were homogeneous with the mass losses after 180 days of wet-and-dry cycles. Figure 3.11 shows the mass losses from PR seeds subjected to constant and fluctuating moisture regimes. The graph illustrates the similarities in mass losses incurred after 90 days at constant moisture and 180 days with fluctuating moisture.

Figure 3.11 Mass loss (%) from *P. radiata* seeds subjected to fluctuating and constant moisture regimes.



Note:

- Error bars indicate standard error of the means.

The net nitrogen released from UE, PR and PC was significantly greater at all time periods under a constant moisture regime. After 180 days, the mineral nitrogen measured in microcosms under constant moisture were approximately 4 times greater than the nitrogen measured from the microcosms under fluctuating moisture. No significant difference between moisture regime was found in the nitrogen released from the NS, NF, CS and SM seeds.

Table 3.29 Summary of two-way ANOVAs testing the main and interaction effects of moisture (constant and wet-and-dry) and time (30, 90 and 180 days) on mass loss and net nitrogen mineralisation from each species.

	Mass loss			N-mineralised		
Species	Moisture	Time	Moisture x Time	Moisture	Time	Moisture x Time
P. radiata	32.25 **	15.29 **	0.01 NS	13.34 *	24.80 **	0.76 NS
P. contorta	74.80 ***	22.09 **	2.89NS	14.00 **	1.66 NS	0.23 NS
N. solandri	4.94 NS	52.82 ***	13.48 **	0.43 NS	7.47 *	2.77 NS
N. fusca	36.37 ***	27.52 ***	1.17 NS	1.23 NS	12.76 **	16.15 **
C. scoparius	39.09 ***	66.93 ***	5.96 *	3.02 NS	3.03 NS	0.24 NS
U. europaeus	5.54 NS	77.28 ***	2.94 NS	124.35 ***	55.79 ***	2.65 NS
S. microphylla	6.77 *	4.44 NS	7.90 *	0.29 NS	2.08 NS	1.65 NS

Note

- F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

The microcosm with intermittent moisture contained significantly less microbial growth than the corresponding microcosms maintained at 60-70% moisture. Seed germination was markedly reduced in all species. The growth of germinated seedlings was effectively arrested in the ensuing drying periods. The seedling remains were withered and colonised by microbes.

3.2.9 Decomposition of litter mixtures

The mass loss and the net nitrogen mineralisation from actual and predicted litter mixtures are presented in Figure 3.12-3.19. The expected values were calculated from the mass losses and nitrogen released from the pure litter microcosms. Table 3.30 shows the decay constants of pure and mixed litters from each species. The main and interaction effects of litter mixtures (expected and observed) and time (30, 90 and 180 days) on decomposition parameters were analysed with two-way ANOVAs. A summary of the ANOVAs is presented in Tables 3.31 and 3.32.

Table 3.30 Decay constants (k) of mass from *P. radiata*, *U. europaeus*, *N. fusca* and *N. solandri* pure and litter mixtures.

Litter type	*P. radiata*	*U. europaeus*	*N. fusca*	*N. solandri*
Seed	0.93	1.16	1.12	1.07
Twig	ND	ND	0.34	0.35
Leaf	0.37	0.88	0.46	0.31
Seed + twig	ND	ND	0.86	0.80
Seed + leaf	0.30	1.02	0.44	0.54
Leaf + twig	ND	ND	0.45	0.30
Seed + twig + leaf	ND	ND	0.45	0.46

Note
ND = not determined.

Table 3.31 Summary of two-way ANOVAs testing the effects and interactions of litter mixtures (expected and observed) and time (30, 90 and 180 days) on mass loss.

Species	Litter mixture	Mixture	Time	Mixture x Time
P. radiata	Seed + leaf	53.76 ***	49.80 ***	5.23 *
U. europaeus	Seed + leaf	6.07 *	57.93 ***	2.63 NS
N. solandri	Seed + twig	29.38 **	382.40 ***	16.12 **
	Seed + leaf	14.00 **	40.45 ***	11.40 **
	Twig + leaf	2.21 NS	35.89 ***	1.78 NS
	Seed + twig + leaf	0.31 NS	26.12 **	2.18 NS
N. fusca	Seed + twig	7.86 *	177.59 ***	10.62 *
	Seed + leaf	9.12 *	303.32 ***	101.52 ***
	Twig + leaf	10.63 *	209.05 ***	9.12 *
	Seed + twig + leaf	110.70 ***	288.87 ***	48.42 ***

Note

- F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

Table 3.32 Snmmary of two-way ANOVAs testing the effects and interactions of litter mixtures (expected and observed) and time (30, 90 and 180 days) on net nitrogen mineralisation .

Species	Litter mixture	Mixture	Time	Mixture x Time
P. radiata	Seed + leaf	57.43 ***	6.27 *	1.82 NS
U. europaeus	Seed + leaf	12.25 **	39.37 ***	11.01 **
N. solandri	Seed + twig	1.41 NS	428.67 ***	7.00 **
	Seed + leaf	38.00 ***	29.55 ***	4.50 *
	Twig + leaf	22.61 ***	20.11 ***	15.75 ***
	Seed + twig + leaf	293.63 ***	213.99 ***	17.31 ***
N. fusca	Seed + twig	15.54 **	17.32 ***	2.24 NS
	Seed + leaf	22.03 ***	10.10 **	2.02 NS
	Twig + leaf	22.61 ***	20.11 ***	15.75 ***
	Seed + twig + leaf	36.95 ***	11.27 **	13.66 ***

Note

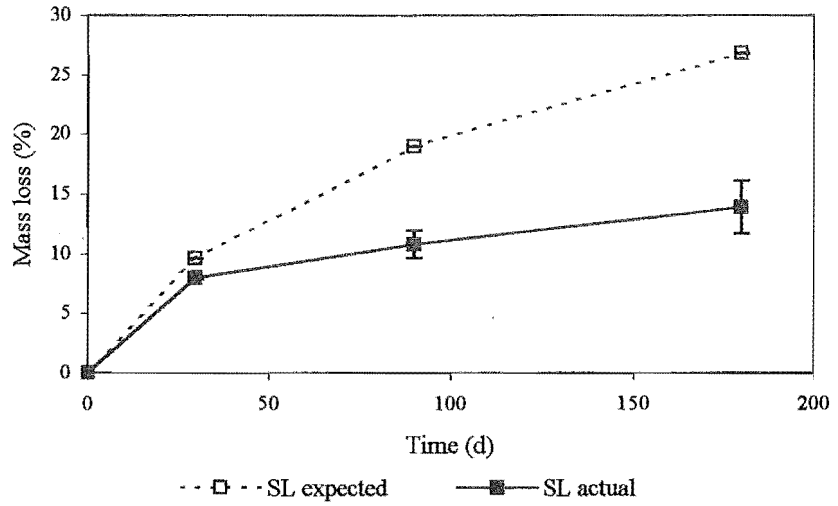
- F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

3.2.9.1 *Pinus radiata*

The mass loss and the net nitrogen mineralised from the actual and predicted litter mixtures, as a function of time, is presented in Figure 3.12 and Figures 3.13, respectively. A large mixture effect was found in the mass loss ($F=53.76$, $P=0.0003$) and net nitrogen mineralisation ($F=57.43$, $P=0.0000$). LSD found the expected and the actual mass losses were homogenous after 30 days. After 90 and 180 days incubation, significantly less mass loss occurred in the mixtures than predicted from the sum of pure litters. The calculated decay constants for mass loss decreased from 0.93 in seeds decomposed in isolation to 0.30 for seeds decomposed in combination with needle material. This value was similar to the pure needle litter decay constant of 0.37.

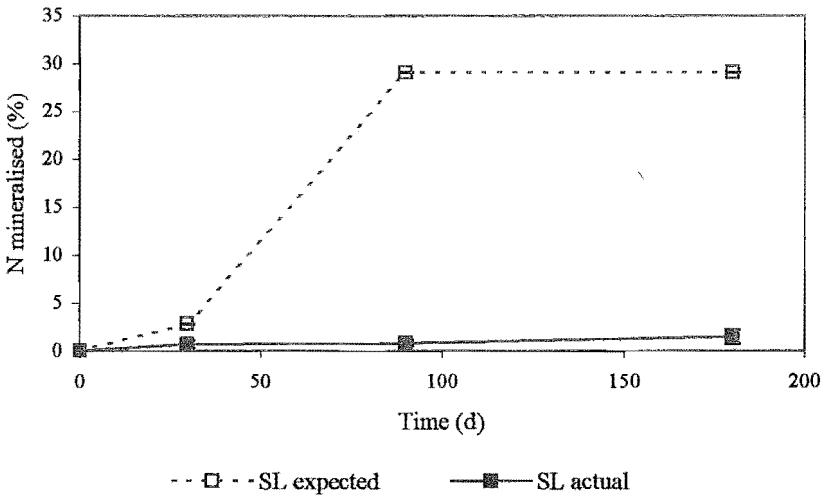
The nitrogen released from the litter mixtures did not vary significantly over all incubation periods. Less than 1.5% net nitrogen mineralisation was recorded at all incubation periods. The expected release of nitrogen greatly exceeded the actual nitrogen release at all time periods, suggesting an antagonistic interaction of litter mixtures.

Figure 3.12 The expected and actual mass loss (%) from mixture of *P. radiata* seed and leaf litters.



Note: Errors bars represent standard error of the mean. SL = seed + leaf.

Figure 3.13 The expected and actual net nitrogen mineralised (%) from mixtures of *P. radiata* seed and leaf litters.



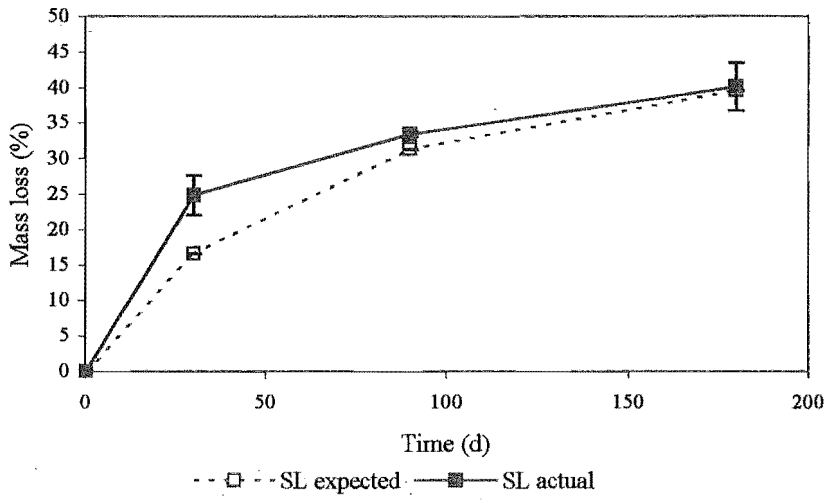
Note: Errors bars represent standard error of the mean. SL = seed + leaf.

3.2.9.2 *Ulex europaeus*

The mass loss and the net nitrogen mineralised from actual and predicted litter mixtures is presented in Figure 3.14 and Figures 3.15 respectively. The decomposition of combined seed and leaf litters had only a minimal effect on litter mass loss (F=6.07, P=0.0489). The calculated decay constants decreased only slightly from 1.16 in pure seeds to 1.02 for seeds in the presence of leaf litter. Significant differences were only found in the early stage of decomposition where litter combinations had a positive effect on mass loss. A significant effect of litter mixtures was found in the measurement of nitrogen release (F=12.25,

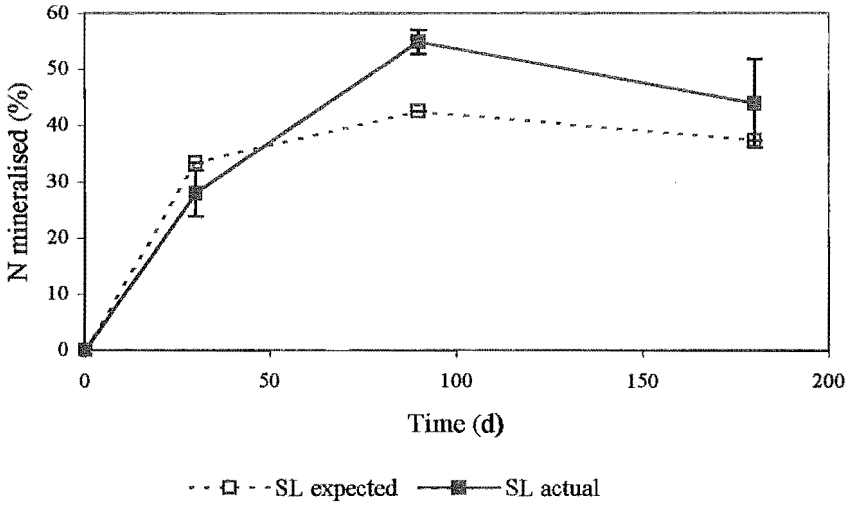
P=0.0044), which exceeded the predicted values. The presence of a significant interaction between time and mixture indicates the non-additive effect of litter mixtures is dependent on the time course of decomposition.

Figure 3.14 The expected and actual mass loss (%) from mixtures of *U. europaeus* seed and leaf litters.



Note: Errors bars represent standard error of the mean. SL = seed + leaf.

Figure 3.15 The expected and actual net nitrogen mineralised (%) from mixtures of *U. europaeus* seed and leaf litters.



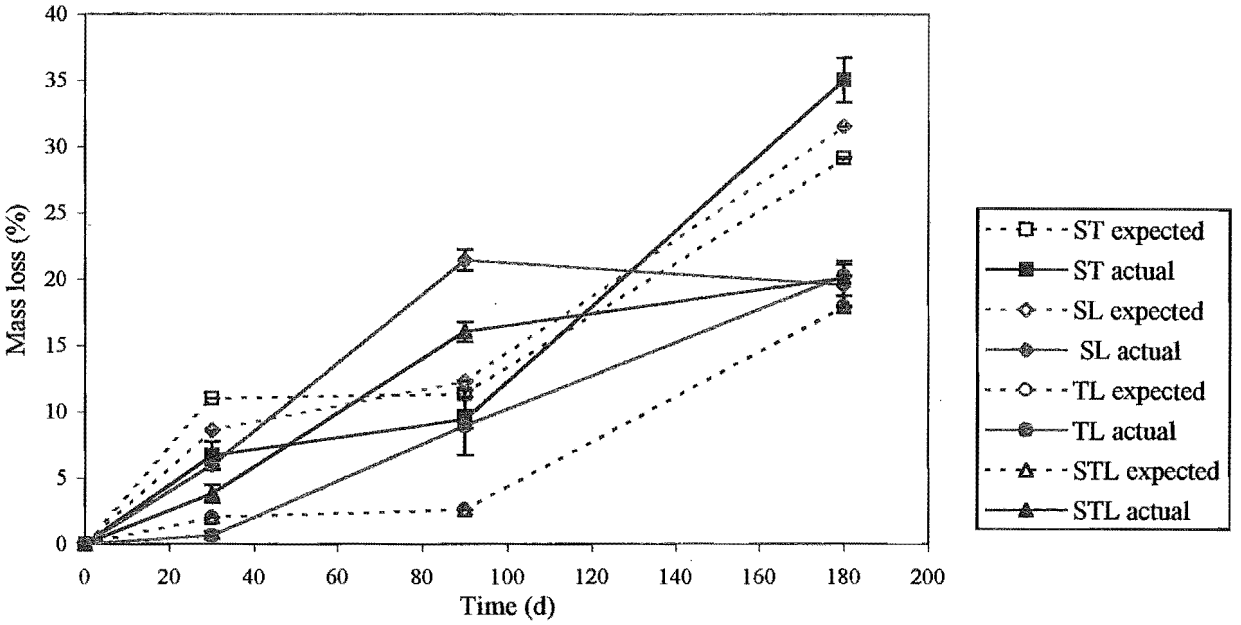
Note: Errors bars represent standard error of the mean. SL = seed + leaf.

3.2.9.3 *Nothofagus fusca*

The mass loss and the nitrogen released from litter mixtures and their predicted values are presented in Figure 3.16 and Figure 3.17. A significant effect of litter mixtures on mass loss was found in all mixture combinations. Overall the actual values were less than predicted for seed + leaf (SL) and seed + twig (ST) litter combinations, and exceeded the predicted values in leaf + twig (LT) and seed + leaf + twig (SLT) combinations. The largest effect of litter combination was found in SLT mixtures. At all time periods the observed exceeded the predicted values, however this was only significant at 90 days, where mass loss deviated from the predicted by 14%.

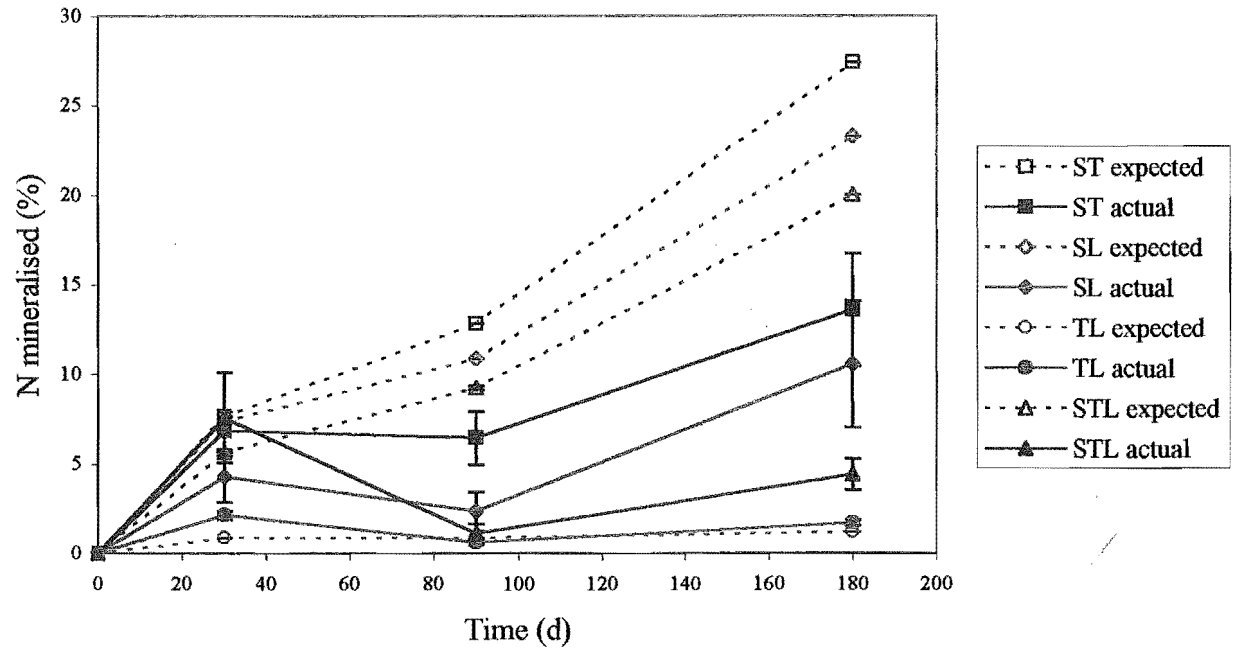
The net nitrogen mineralisation was significantly reduced in ST, SL and STL litter combinations. The LSD test found the means of expected and actual values homogeneous at 30 days. However, significant differences were found at 90 and 180 days, indicating an antagonistic effect of litter mixtures. The predicted and observed nitrogen release in ST and SL did not significantly vary between 30 and 90 days. A significant increase in nitrogen release was measured at 180 days. The nitrogen mineralisation from STL showed an initial release in first 30 days followed by a dominance of immobilisation at 90 and 180 days.

Figure 3.16 The expected and actual mass loss (%) from mixtures of *N. fusca* seed, twig and leaf litters.



Note: Errors bars represent standard error of the mean. ST= seed + twig, SL = seed + leaf, TL= twig + leaf, STL = seed + twig + leaf.

Figure 3.17 The expected and actual net nitrogen mineralisation (%) from mixtures of *N. fusca* seed, twig and leaf litters.



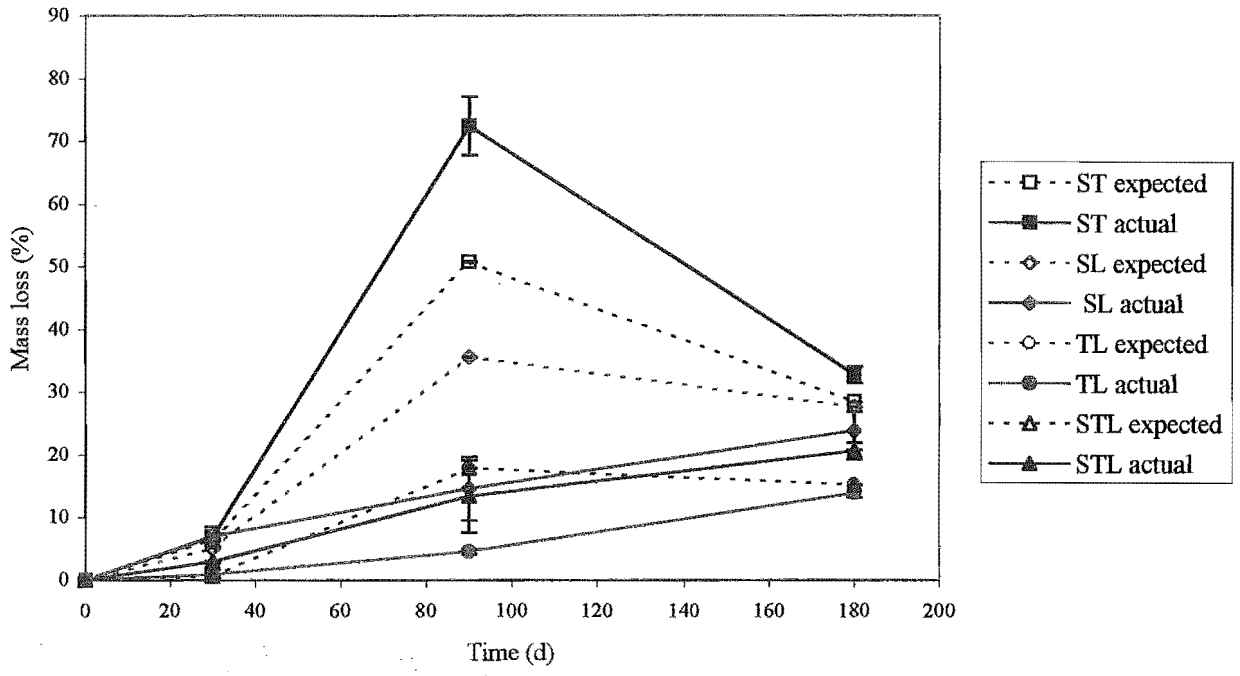
Note: Errors bars represent standard error of the mean. ST= seed + twig, SL = seed + leaf, TL= twig + leaf, STL = seed + twig + leaf.

3.2.9.4 *Nothofagus solandri*

The mass loss and net nitrogen mineralisation from mixtures of *N. solandri* litter is shown in Figure 3.18 and Figure 3.19 respectively. A significant difference between the expected and the actual results were only found in seed + twig (ST) and seed + leaf (SL) mixtures. The mass loss at 90 days from ST and pure seed microcosms were erroneously large. The measurements from pure seed microcosms were used to predict mass losses in mixtures containing seeds. As a consequence the error was continued into the predicted values at 90 days. The ST, pure seed and pure twig microcosms were set up and analysed at the same time. The experimental error is consistent in each of these microcosms. Therefore, comparison was valid in the ST mixture but invalid in SL and STL mixtures. The exclusion of the 90 day values from SL, ST and STL resulted in no significant difference between the expected and actual mass loss.

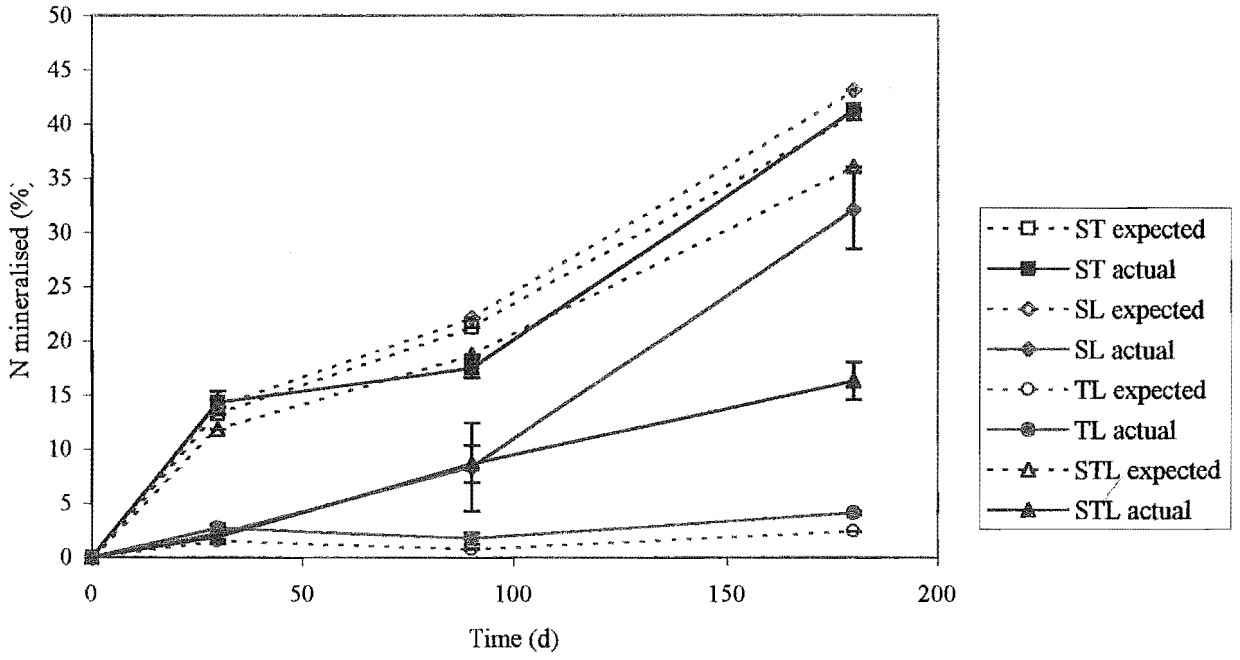
The actual nitrogen released from ST and LT mixtures closely paralleled the predicted nitrogen release. In the ST mixture the nitrogen released at 90 days significantly deviated from predicted nitrogen release. At 30 and 180 days the values of ST were homogeneous. LT expected and actual appears very similar in Figure 3.19. However, the ANOVA found the observed significantly exceeded the predicted nitrogen mineralisation at each time period. The nitrogen released from the SL and STL litter mixtures was consistently lower than predicted at each time period.

Figure 3.18 The expected and actual mass loss (%) from mixtures of *N. solandri* seed, twig and leaf litters.



Note: Errors bars represent standard error of the mean. ST= seed + twig, SL = seed + leaf, TL= twig + leaf, STL = seed + twig + leaf.

Figure 3.19 The expected and actual net nitrogen mineralisation (%) from mixtures of *N. solandri* seed, twig and leaf litters.



Note: Errors bars represent standard error of the mean. ST= seed + twig, SL = seed + leaf, TL= twig + leaf, STL = seed + twig + leaf.

3.3 *IN SITU* DECOMPOSITION

3.3.1 Decomposition of whole seeds at 1 cm and 10 cm depth

The net mass and nitrogen losses from whole seeds buried at depths of 1 cm and 10 cm, for 30, 90 and 180 days, are presented in Table 3.33 and Table 3. 34. The percentage mass was calculated on an oven dry, ash free basis. Nitrogen loss represents the absolute change in nitrogen as a percentage of initial seed nitrogen, expressed on an oven dry, ash free basis. A two-way ANOVA was performed on each species. The analysis tested for significant differences in mass and nitrogen loss between soil depth over time. A summary of the ANOVAs is presented in Table 3.35.

Table 3.33 Mass loss (%) and decay constants (k) from seeds incubated *in situ* at 1 cm and 10 cm depth for 30, 90 and 180 days.

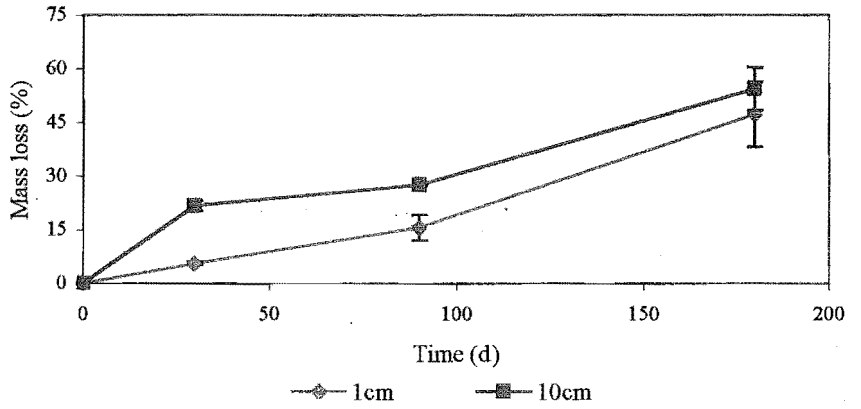
Species	Depth	30d (n=2)	90d (n=2)	180d (n=2)	k (0-180 days)
P. radiata	1 cm	8.7 ± 0.8	13.2 ± 4.6	71.0 ± 0.3	2.45 ± 0.00
	10 cm	7.9 ± 1.0	11.3 ± 1.3	71.4 ± 0.3	2.50 ± 0.00
P. contorta	1 cm	3.5 ± 0.4	15.8 ± 1.8	46.8 ± 17.1	1.37 ± 0.04
	10 cm	13.6 ± 5.4	34.9 ± 7.1	60.3 ± 2.1	1.84 ± 0.01
N. fusca	1 cm	4.0 ± 1.8	14.6 ± 1.2	54.6 ± 15.0	1.70 ± 0.04
	10 cm	1.8 ± 0.1	25.3 ± 0.9	58.2 ± 4.4	1.76 ± 0.01
N. solandri	1 cm	5.5 ± 1.4	15.6 ± 0.3	47.3 ± 6.0	1.29 ± 0.01
	10 cm	21.6 ± 0.5	27.5 ± 3.5	54.4 ± 9.0	1.61 ± 0.02
U. europaeus	1 cm	11.7 ± 3.0	50.2 ± 3.0	73.8 ± 2.4	2.69 ± 0.01
	10 cm	14.1 ± 0.1	55.3 ± 1.4	61.7 ± 4.5	1.93 ± 0.01
C. scoparius	1 cm	20.3 ± 15.0	10.5 ± 0.7	17.1 ± 4.5	0.38 ± 0.01
	10 cm	10.6 ± 3.8	8.6 ± 0.2	18.5 ± 11.1	0.43 ± 0.02
S. microphylla	1 cm	0.1 ± 0.1	0.4 ± 0.3	0.4 ± 0.3	0.01 ± 0.00
	10 cm	0.0 ± 0.0	1.2 ± 0.4	0.5 ± 0.5	0.01 ± 0.00
Q. robur	1 cm	0.0 ± 0.0	10.0 ± 1.0	37.1 ± 10.3	0.96 ± 0.02
	10 cm	0.0 ± 0.0	31.1 ± 5.0	20.5 ± 8.0	0.47 ± 0.01

Note
- Figures are presented as mean ± standard error of the mean.
- n = number of replicates.

Mass loss increased over time for all species with the exception of CS (F=0.48, P=0.6433) and SM (F=0.76, P=0.5060). In the *Nothofagus* and *Pinus* species the rate of mass loss increased substantially between 90 and 180 days. LSD on each species found the values from both soil depths to be homogeneous at 30 and 90 days incubation. A significant

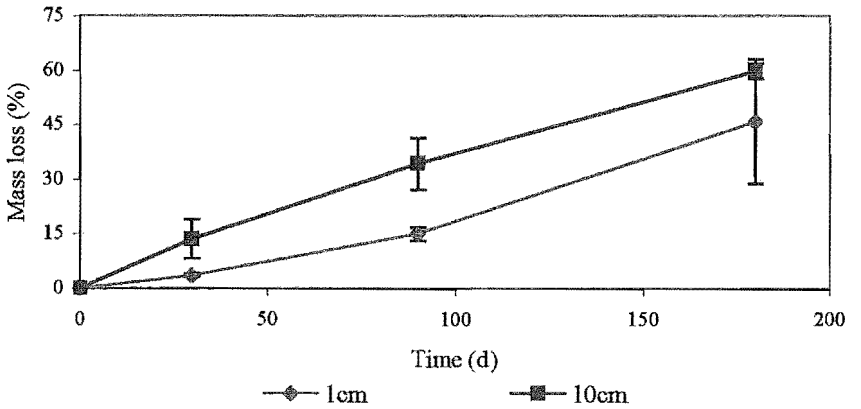
difference in mass loss between depth was only found in NS (Figure 3.20). The losses incurred at 10 cm exceeded the mass loss at 1 cm at all time periods. The mass loss varied between depths by 16%, 11% and 7% after 30, 90 and 180 days incubation respectively. A similar pattern was observed with PC seeds, as shown in Figure 3.21. However, this was found to be non-significant (F=4.81, P=0.0707). For PR, QR, UE and NF the greatest variability was associated with the period of incubation, followed by the residual error in the ANOVA. The large residual error was a consequence of the large variability found between replicates.

Figure 3.20 Mass loss (%) from *N. solandri* seeds *in situ* at 1 cm and 10 cm depth.



Note
- Error bars indicated refer to standard error of the means.

Figure 3.21 Mass loss (%) from *P. contorta* seed *in situ* at 1 cm and 10 cm depth.



Note
- Error bars indicated refer to standard error of the means.

Extensive mass losses were observed after 180 days for PR, PC, NS, NF and UE. Greater than 80% mass loss, within the first year, was predicted for PR, PC, NF and UE, illustrating the rapid turnover of seed substrates *in situ*.

The pattern of nitrogen loss was complicated and varied substantially between species (Table 3.34). The quantity of nitrogen released generally increased with time for NS, NF, UE, PR and PC. For these species, the quantity of nitrogen loss paralleled the loss of seed mass. The seeds of CS and SM experienced an initial stage of nitrogen loss followed by movement of nitrogen into the seed. The nitrogen loss from the seeds showed no significant difference with soil depth for all species. CS nitrogen loss at 30 days varied by 11 % between each depth, however LSD found the means homogeneous, due to the large variability between replicate litter bags. The mean nitrogen loss from QR seeds was homogenous for all time periods with the values varying by only 7 %. The results of QR must be interpreted with caution, as only two seeds were included in each litter bag due to the large seed size. The small number of seeds within each litter bag, increases the influence of individual variability.

Table 3.34 The nitrogen loss from seed *in situ* at 1 cm and 10 cm depth for 30, 90 and 180 days.

Species	Depth	30d (n=2)	90d (n=2)	180d (n=2)
P. radiata	1 cm	12.3 ± 3.9	17.1 ± 5.0	93.9 ± 0.2
	10 cm	14.3 ± 1.8	11.0 ± 3.0	93.3 ± 0.7
P. contorta	1 cm	0.6 ± 0.4	4.1 ± 0.3	49.5 ± 22.6
	10 cm	7.9 ± 9.2	34.0 ± 10.0	72.9 ± 1.8
N. fusca	1 cm	22.5 ± 2.7	20.3 ± 2.1	59.7 ± 14.1
	10 cm	27.1 ± 1.2	25.9 ± 2.9	63.9 ± 1.0
N. solandri	1 cm	9.5 ± 0.7	15.5 ± 2.9	45.3 ± 0.8
	10 cm	28.5 ± 4.3	17.5 ± 4.1	45.9 ± 18.2
U. europaeus	1 cm	19.4 ± 2.6	46.4 ± 0.3	73.5 ± 0.7
	10 cm	15.6 ± 3.4	58.5 ± 5.4	64.2 ± 6.5
C. scoparius	1 cm	27.5 ± 12.6	6.8 ± 3.2	14.3 ± 11.3
	10 cm	16.3 ± 3.2	8.8 ± 0.9	19.0 ± 11.8
S. microphylla	1 cm	13.1 ± 2.2	-13.6 ± 0.7	-18.4 ± 0.6
	10 cm	-0.8 ± 0.7	-3.7 ± 1.2	-16.1 ± 4.2
Q. robur	1 cm	12.4 ± 4.4	11.5 ± 7.2	9.4 ± 29.1
	10 cm	14.6 ± 5.6	10.9 ± 14.2	7.3 ± 9.1

Note

- Figures are presented as mean ± standard error of the mean.
- n = number of replicates.

Table 3.35 Summary of two-way ANOVAs testing the effects and interaction of soil depth (1 cm and 10 cm) and time (30, 90 and 180 days) on nitrogen loss (%) and mass loss (%).

	Mass loss			N loss		
Species	Depth	Time	Depth x Time	Depth	Time	Depth x Time
P. radiata	0.21 NS	605.59 ***	0.17 NS	0.22 NS	53.87 ***	1.10 NS
P. contorta	4.81 NS	16.34 **	0.17 NS	3.49 NS	8.71 *	0.78 NS
N. solandri	9.22 *	34.74 ***	0.45 NS	1.23 NS	8.31 *	0.83 NS
N. fusca	0.57 NS	35.66 ***	0.50 NS	2.84 NS	36.41 ***	0.16 NS
C. scoparius	0.25 NS	0.48 NS	0.02 NS	0.05 NS	1.37 NS	0.49 NS
U. europaeus	0.46 NS	207.77 ***	5.53 *	0.01 NS	91.14 ***	4.07 NS
Q. robur	0.45 NS	80.10 ***	1.34 NS	0.00 NS	0.07 NS	0.01 NS
S. microphylla	1.77 NS	0.76 NS	0.52 NS	0.11 NS	67.77 ***	17.77 **

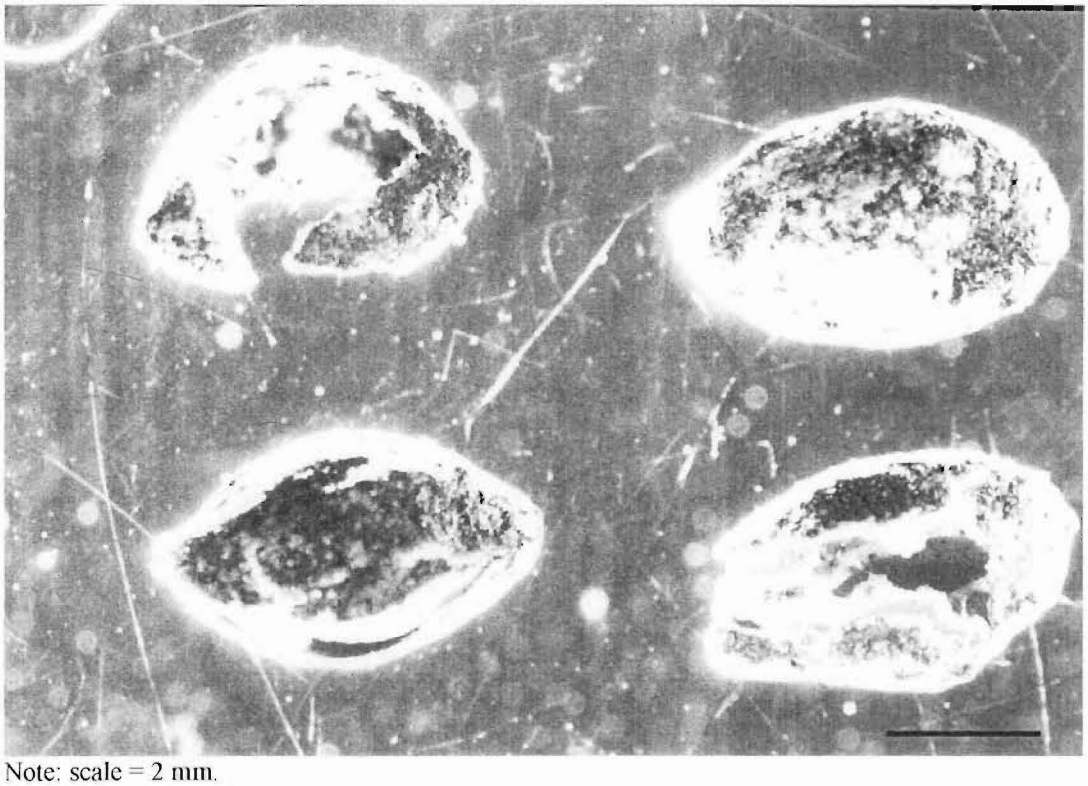
Note

- F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

The incubation of seeds resulted in the discolouration, microbial colonisation and fragmentation in some species. After 180 days the *Pinus* and *Nothofagus* seeds were devoid of endosperm, with only the dull seed coats remaining. In some replicate litter bags the seeds appeared highly fragmented. Microscopic examination revealed the presence of insect larva within the litter bag that may have contributed to the extensive seed fragmentation. Figure 3.22 shows the damaged PC seeds after *in situ* burial. The fragmentation and presence of larva was localised and not consistently present in all replicates, consequently increasing the variability within treatments. No microbial growth was observed on SM and only minimal growth on CS. The seeds of UE lost their lustre over time. Some UE seeds appeared macerated and obvious microbial growth was present. In all species the seed status and their corresponding microbial growth showed large variation between replicates.

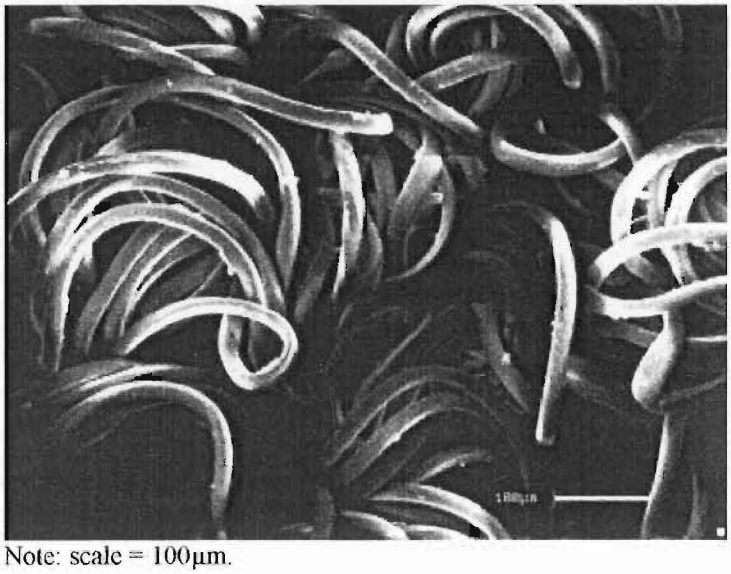
The nylon bags recorded minimal changes in weight. Electron micrographs of nylon mesh showed no degradation of nylon. A comparison of incubated nylon and control nylon showed the presence of organic material. This quantity of extraneous material was minimal as shown in Figure 3.23.

Figure 3.22 Fragmented seeds of *P. contorta* after 90 days incubation *in situ*.



Note: scale = 2 mm.

Figure 3.23 Nylon mesh of litter bag after 180 days incubation *in situ*.



Note: scale = 100µm.

The mass losses from whole seeds at 1 cm depth in the soil were compared with the corresponding values from whole seeds incubated in microcosms with temperatures approximating diurnal cycles (5°C -15°C). The *in vitro* and *in situ* data were statistically analysed using two-way ANOVA. The treatments of incubation conditions and time were tested for their effects and interactions on seed mass loss. A summary of the ANOVAs is presented in Table 3.36.

A significant difference between mass losses from microcosm and litter bag experiments was found in CS, UE, NS and PR. The litter bag results of CS exceeded those obtained from microcosms at all time periods. In UE and PR the mass loss from litter bags was greater than in microcosms. The difference increases markedly over time. Minimal differences were measured during the early stages, increasing to over 30% after 180 days. NS incurred greater mass losses in the microcosms, with the mean value for all time periods of incubation equalling 27.7% for *in vitro* experiments and 22.8% for *in situ* experiments. No significant difference between microcosm and litter bag results were found for the remaining species.

Table 3.36 Summary of two-way ANOVAs testing the effects and interactions of incubation conditions (*in vitro* and *in situ*) and time (30, 90 and 180 days) on mass loss (%).

Species	Condition	Time	Condition * Time
P. radiata	20.36 **	100.05 ***	25.20 ***
P. contorta	1.17 NS	34.46 ***	1.22 NS
N. solandri	8.37 *	162.66 ***	32.01 ***
N. fusca	3.24 NS	111.70 ***	2.35 NS
C. scoparius	9.37 *	2.35 NS	7.93 NS
U. europaeus	85.76 ***	227.03 ***	30.19 ***
Q. robur	2.55 NS	9.11 **	6.22 *
S. microphylla	8.87 NS	16.83 NS	13.17 NS

Note

- F values are displayed with significance denoted by P<0.05=*, P<0.01=**, P< 0.001=***, NS = not significant.

3.3.2 Decomposition of heat-treated seeds on the soil surface

The percentage mass loss and nitrogen loss after 180 days, expressed on an oven dry, ash free basis is presented in Table 3.37. The calculated decay constants are also included in Table 3.37. The mass loss varies significantly between species over all time periods (F=21.00, P=0. 0000). The pooling of data from all species gave a mean value of 4.0%, 8.7% and 21.2% mass loss for 30, 90, and 180 days respectively. LSD test of the means found no significant difference in mass losses after 30 and 90 days incubation. The HV, TA, NF, QR and SM incurred a large loss of mass between 90 and 180 days, which was greater than that experienced in the first 90 days.

The loss of mass from seeds ranged from 7.5% in NF to 62.0% in UE. The net loss of nitrogen occurred in a majority of the test species, ranging from 10.1% in QR to 67.3% in UE. The seeds of PC, NF and NS measured a net increase in nitrogen after 180 days incubation. In all cases only minimal mass losses had occurred from the seeds.

Table 3.37 Mass loss (%), nitrogen loss (%) and decay constants (k) from heat-treated seeds after 180 days incubation on the soil surface.

Species	Mass loss (%) n=3	Nitrogen loss (%) n=3	Mass loss k (0-180)
T. aestivum	30.0 ± 6.3	20.2 ± 6.1	0.71
H. vulgare	17.3 ± 3.4	13.2 ± 4.0	0.38
P. radiata	8.2 ± 2.8	6.5 ± 4.9	0.17
P. contorta	3.3 ± 1.6	-6.1 ± 1.7	0.07
N. solandri	14.8 ± 1.8	-6.8 ± 4.0	0.32
N. fusca	7.5 ± 2.4	-4.1 ± 6.3	0.16
C. scoparius	60.0 ± 8.9	61.9 ± 7.2	1.83
U. europaeus	62.0 ± 1.8	67.3 ± 3.6	1.94
S. microphylla	33.0 ± 16.9	18.2 ±9.8	0.80
Q. robur	3.0 ± 0.0	10.1 ± 2.8	0.06

Note
- Figures are presented as mean ± standard error of the mean.
- n = number of replicates.

The differences between mass loss from *in vitro* and *in situ* experiments were statistically analysed. Two-way ANOVAs investigated the effect and interaction of incubation conditions (*in vitro* and *in situ*) and time (30, 90 and 180 days) on seed mass loss. In all species, the microcosm experiments incurred greater mass losses than seeds incubated *in*

situ. However, the differences were not significant in CS, UE and PR varying by only 8%, 6% and 0.3% respectively. In the HV, TE, SM and NF species, the difference increased over time to greater than 30% after 180 days incubation. The species ranked in accord with mass loss after 180 days was very similar to that obtained from microcosm experiments (Table 3.11). The only difference occurs in the ranking of SM and CS. Greater mass loss was recorded for CS *in situ* than SM, whereas the inverse was true for the microcosm experiments.

The *in situ* results of whole and heat-treated seeds, over time, were compared using two-way ANOVAs. With the exception of UE, all species mass losses were significantly different between the seed treatments. CS and SM heat-treated seeds lost significantly more mass than the whole seeds. The remaining species measured greater mass losses from whole seeds over all time periods. Preponderantly the differences between whole and heat-treated seeds were amplified with time in the soil.

3.3.3 Decomposition of seeds in three different soils

The mass loss from whole seeds of PR, PC, NF, NS, UE and CS incubated in three different soils for 180 days, is displayed in Table 3.38. A pH of 6.8, 6.7 and 5.7 was determined for the Darfield, Lincoln and Hawdon soils respectively. All seeds showed evidence of decomposition after 180 days. The *Nothofagus* species showed extensive fragmentation and damage to the seeds in all soils. The *Pinus* species were dull and devoid of endosperm in the Darfield and Lincoln soils. However, the seeds incubated in the Hawdon soil still contained endosperm. In CS and UE the residual number of seeds in the litter bags, after 180 days incubation, were significantly reduced in comparison to day zero. The remaining whole seeds had retained their lustre and showed no signs of microbial colonisation.

The mass loss varied significantly between species. Approximately 36% of the variation between all pooled data was attributed to seed species. The mean for all soil types ranged between 61.0% in NS and 49.1% in PR. One-way ANOVA was performed on each species to determine the effect of soil type on mass loss. Soil type was only significant in PC ($F=113.3$ $P=0.0000$) and CS ($F=15.84$, $P=0.0040$). The mass loss from the Hawdon soil was significantly less than the Lincoln and Darfield soils in both species. PR seeds also showed a reduction in mass loss in the Hawdon soil, however this was only determined to be significant by LSD. PR seeds showed a considerable variability between replicates, indicated by the large standard errors. This large variability within a soil type would have contributed to the non-significant effect found by the ANOVA ($F=4.72$, $P=0.0586$).

Table 3.38 Mass loss (%) and decay constants (k) from whole seeds in Hawdon, Darfield and Lincoln soil.

	Soil Type		
Species	Hawdon n=3	Darfield n=3	Lincoln n=3
P. radiata	25.2 ± 14.0 (0.58)	52.9 ± 10.9 (1.51)	69.4 ± 1.6 (2.37)
P. contorta	31.1 ± 2.3 (0.75)	58.4 ± 1.2 (1.76)	60.9 ± 0.9 (1.88)
N. fusca	57.6 ± 3.6 (1.72)	59.7 ± 0.7 (1.82)	56.8 ± 3.8 (1.68)
N. solandri	59.2 ± 5.5 (1.79)	65.3 ± 2.9 (2.12)	58.7 ± 3.3 (2.12)
U. europaeus	30.7 ± 3.9 (0.73)	27.3 ± 5.0 (0.64)	33.4 ± 4.1 (0.81)
C. scoparius	37.0 ± 2.9 (0.92)	64.5 ± 3.4 (2.07)	61.6 ± 3.4 (1.91)

Note: Figures are presented as mean ± standard error of the mean. n = number of replicates. Values in parentheses indicate the decay constants (k).

4. DISCUSSION

4.1 The seed component of plant litter

Large quantities of energy and nutrients continually circulate between the above-ground and below-ground components of the terrestrial ecosystem. Comprehending this transfer of nutrients and energy between these components is fundamental to understanding ecosystem functions and processes. Numerous studies have attempted to model the input, output and transfer of nutrients in the ecosystem with various nutrient and energy budgets. Commonly, these budgets estimate that the nutrient demand of the above-ground components exceeds the supply capacity of the soil, thereby leaving the system in deficit (Heal *et al*, 1982). Such discrepancies have been attributed to inaccuracies in quantifying the inputs of organic material into the soil from primary and secondary sources.

The periodic release of energy- and nutrient-rich seeds is a fraction of the plant litter that is often excluded. Omission of this component due to the periodicity of seed release or the assumption that the component is quantitatively insignificant can result in substantial underestimates of the energy and nutrients entering the soil system (Ovington, 1963; Carlisle *et al*, 1966; Proctor *et al*, 1983; Stocker *et al*, 1995). Estimates of the proportional contribution of reproductive material to the total litter deposition can range from 3-37% and 4-61% of the total litter biomass and nitrogen respectively (refer to Table 1.1).

Assuming the seeds are physiologically equipped for germination, the value of the seeds in reproduction will vary temporally and be spatially related to the availability of suitable microsites for germination. If the appropriate biotic and abiotic conditions of the environment are available, the seeds may potentially establish and therefore is not considered a loss to the plant. Alternatively, the absence of suitable microsites effectively renders all seed material, prior to its production and release, detrital litter. The potential reproductive value of the seed material complicates quantification of the seed litter fraction. In this study all seeds released from the parent are considered a component of the litter. The reproductive fraction of the seed lot is recognised, but considered incidental for the purpose of this study.

Despite any reproductive value, only a fraction of the seeds released will germinate and establish as individual seedlings. By deductive logic, a large portion of the seeds and their associated nutrients will enter the detrital pathway, either directly following release or indirectly via predator activity. Nielsen (1977) tentatively estimated that 95% of *Fagus sylvatica* seeds were entering the detrital pathway annually. This represents an input of up to 1.8 t $ha^{-1}yr^{-1}$ of seed material and an energy flux of 1150 kJ $m^{-2}yr^{-1}$.

The release of seeds into the soil system quantitatively represents a large flux of energy and nutrients into the soil. The availability of the seed nutrients and energy to the soil heterotrophic community is relatively uncertain. An array of circumstantial evidence indicates a microbial fate of the subterranean seeds. This evidence includes:
1) Microbes function as a mechanism of seed scarification through the degradation of the seed coat (Gogue & Emino, 1979; van Leeuwen, 1981; Crist & Friese, 1993).
2) Germination studies have reported a reduced viability with exposure to the soil matrix and its associated decomposer community (Burrows, 1991; Burrows, 1993; Alvarez-Buylla & Martínez-Ramos, 1990).
3) Soil seed bank studies have found a reduction in seed loss in the presence of fungicidal agents (Lonsdale, 1993; Jansen & Ison, 1995; Dalling *et al*, 1998; Leishman *et al*, 2000).
4) The fertilisation effect of dead seeds on proximate seedling growth implies the nutrients of seeds may be rapidly released and available for plant uptake (Zackrisson *et al*, 1999).

Despite these numerous inferences of microbial decomposition of seeds in the soil, limited biochemical research on this subject has been undertaken. This study, in agreement with the work of Greenfield (1998), provides direct biochemical evidence of the rapid mobilisation of seed nutrients by microorganisms. The rate of release is primarily dependent on the substrate quality. This is determined by the collective physical and chemical properties of the seed coat and embryo-endosperm fractions.

4.2 Characterisation of seed substrates

The ten test species in the study are divergent with respect to their chemical and physical properties. The dry weight of seeds varied between species, ranging from 4.76 mg in *P. contorta* to 2.32 g in *Q. robur*. The values obtained are consistent with mass values noted in other studies (Harper & Lynch, 1980; Ledgard & Cath, 1983; Wardle, 1984; Williams, 1981; Menzies *et al*, 1991; Jinadasa, 2000).

The pH, ash and moisture figures were relatively consistent between the species. The seeds surveyed had a mean pH 5.7, conforming with the general acidity of plant litter (Mason, 1977). The mean ash content for all species in this study measured 3.2% (range 1.5-7.1%). The ash values of *N. solandri*, *N. fusca* and *S. microphylla* species collected from the litter layer, were not higher than the other species, indicating minimal soil contamination in the collection method employed. Moisture content of all species varied between 8-14% of the seeds' fresh weight. These values are within the 4-16% range for dry seeds specified by Priestley (1986). This suggests the seeds were stored at an adequately low moisture content to retard biochemical degradation processes that promote a loss of seed viability (Abdul-Baki & Anderson, 1972; Copeland & McDonald, 1995).

The water retention in seeds diverged significantly between 18% in *S. microphylla* to 258% in *N. fusca*. The large values found in the *Nothofagus* species were comparable to the water retention found in leaf litter. The water retention of broken *Picea*, *Pinus* and *Populus* leaf litter amounted to 214%, 221% and 371% respectively (Daubenmire & Prusso, 1963). The large water retention in the *Nothofagus* species may be attributed to the triquestrous shape of a proportion of the *Nothofagus* seeds, thereby increasing the surface area exposed to water. Low water retention in the legume species can be ascribed to the hard impervious seed coat, characteristic of this family. However, the seeds of another legume species, *Arachis hypogeal*, showed a 152% increase in seed mass after seed hydration (Samad & Pearce, 1978). This is comparatively greater than the values found for legumes in the present study, presumably due to the atypically thin testa of *A. hypogeal*.

Leaf litter also exhibits variation between species with respect to water retention. Walsh and Voigt (1977) found *Fagus sylvatica* leaf litter was able to retain 1.5 times more water than

Pinus sylvestris. Differential water retention between species has a regulating effect on the microbial communities. Species with high water potential are generally colonised by bacteria, whereas species with low water potential are predominantly colonised by fungi and Actinomycetes (Howard & Howard, 1980).

A significant negative correlation was found between seed water retention and the quantity of water-soluble extractives in ground seed material. This may be a consequence of the simultaneous opposing action of water uptake and retention increasing the seed mass, and the leaching of water-soluble material reducing the seed mass (Simon & Raja Harun, 1972).

The nitrogen concentration of the 10 test species ranged from 0.9% in *Q. robur* to 5.8% in *U. europaeus,* with a mean of 3.4%. This mean nitrogen concentration is comparable to the value of 3.0% obtained by Lang and Forman (1978) in a mixed oak stand and similar to the range measured by Greenfield (1998), which varied between 0.9% and 5.3%. In the *P. radiata*, *P. contorta* and *U. europaeus* species, the two populations of seeds sampled showed slight differences in nitrogen concentration. This can be attributed to the different collection sites and the corresponding conditions experienced by plant populations. Similar proportional differences between sites have been observed in other litter fractions. For example, the nitrogen concentration of *Fagus sylvatica* leaf litter varied from 12 mg g^{-1} in a mull site to 10 mg g^{-1} in a mor site (Vesterdal, 1999). The variation in nitrogen concentration between seed populations were not statistically significant suggesting this seed trait is largely under genetic control (Copeland & McDonald, 1995).

A negative correlation of decreasing nitrogen concentration with increasing physical seed dimensions (mass, length and width) was found in this investigation. This trend was also reported by Lee and Fenner (1989) in *Chinochloa* genus and by Grubb and Coomes (1997) in Amazonian caatinga shrub species. Lee and Fenner (1989) postulated the increased nitrogen content of the smaller seeded species may confer some advantage in their reproductive strategy. Increased nitrogen content in smaller seeds may be a reflection of the plants' perceived limiting conditions for seedling establishment. Evidence for such a relationship is often contradictory and it is beyond the scope of this study to speculate on an ecological significance of this association.

The mean nitrogen distribution of seed ammonium, α-amino acid, acid insoluble and hydrolysable unidentified nitrogen fractions were 10%, 45%, 4%, 41% respectively. The proportion of ammonium nitrogen, derived from amide-nitrogen and the breakdown of amino acids during hydrolysis, is consistent with other litter components (Greenfield, 1979; Wardle & Greenfield, 1991). The hydrolysable unidentified fraction (HUN) greatly exceeds the 10-20 % found in leaf litters (Greenfield, 1979). Equivalent proportions of pollen (Greenfield, 1999) and root nodule (Wardle & Greenfield, 1991) nitrogen was fractioned as HUN. The high value of HUN is largely derived from non-α amino acid-N present in amino acids and imino-N in the plant material (Greenfield 1972; Greenfield, 1979; Kelley & Stevenson, 1996). The combined HUN, α-amino acid-N, and ammonium-N, indicates approximately 96% of the nitrogen within the seed is present, as amino acids in a free or proteinaceous form. This high protein content is characteristic of seeds and confers the nutritive value of seed substrates (Bewley & Black, 1978).

The acid insoluble fraction is less than the 20-35% present in leaf litter and soil (Greenfield, 1979; Stevenson, 1982). The components of the acid insoluble fractions are largely unknown. In the past, the acid insoluble fraction was thought to be an artefact of the hydrolysis procedure caused by the condensation of amino acids with reducing sugars. However, more rescent reseach would indicate that the fraction represents the structural nitrogen components (Kelley & Stevenson, 1996). The comparatively reduced structural component in seed material may explain this reduced acid insoluble fraction.

The lipid content varied between 0.2% in *T. aestivum* to 32.1% in *P. radiata*. Similar divergence between species was found in the 14 species surveyed by Greenfield (1998), where the lipid content ranged from 2% to 26% of the seeds' oven dry mass. A large lipid content was found in both *Pinus* species, comprising 28% and 32% of the seeds oven dry mass in *P. contorta* and *P. radiata* respectively. These values are consistent with the measured oil content in *Pinus densiflora* seeds. The oil content was approximately 35% of the seed dry weight (Bewley & Black, 1978). Lipids are the principle storage material in smaller seeds (Copeland & McDonald, 1995), however this study found only a weak non significant correlation between lipid content and seed mass ($r=-0.4851$, $P=0.1553$). The weak relationship may be an artefact of both the small number of species surveyed and the predominance of legume species in this study.

The germination capacity of *H. vugare*, *P. radiata*, *N. solandri* and *N. fusca* concurred with other studies as illustrated in Table 4.1. The remaining species showed a lower germination capacity compared to previous studies. This lowered germinability may be attributed to the age of the seeds, the different collection sites used and the numerous viability tests employed by researchers. It is likely that a combination of these factors is responsible for the germinability discrepancies between studies.

The germination trials in the present study were performed five months following seed collection, thereby reducing the ecological value of the trial. Viability tends to decrease exponentially with time, as structural organisation degrades (Priestley, 1986; Baskin & Baskin, 1998). No comparative germination trials of fresh and stored seeds were performed in this study to account for any temporal decline in viability. Circumstantial evidence of a decline in viability over time was provided by the comparatively higher germination rates observed in the microcosm experiments of *Q. robur* and *U. europaeus* (1-2 months following seed collection) as opposed to the reduced rate in the later germination trials. This was most evident in the seeds of *Q. robur*. The *Quercus* seeds are desiccation sensitive and rapidly lose viability if seed moisture is below 30-65% (Baskin & Baskin, 1998). The rapid loss of viability in these recalcitrant seeds is speculated to involve the unregulated metabolism and the concomitant accumulation of toxins and free radicals. These conditions can potentially cause membrane damage (Finch-Savage *et al*, 1996). A comparable low germination rate has been recorded in other *Quercus* species. Steele *et al* (1993) measured 4% germination in the whole seeds of *Q. phellos*.

Sterilisation can remove microbial substrates and microbial communities (Harper & Lynch, 1980), the presence of which may play an important role in the inhibition and promotion of germination (Kirkpatrick & Bazzaz, 1979; Kremer, 1986b). The germination trials of the present study were performed under non-sterile conditions. As a consequence, extensive fungal growth was observed in all germination trials. This growth may have contributed to the reduced viability. The non-sterile conditions employed in this study were believed to be of greater ecological relevance to germination potential in the field, considering the ubiquitous distribution of microorganisms.

Table 4.1 Seed viability.

Species	Viability (%) present study	Viability (%) and reference
H. vulgare	99.3	>90 Harper & Lynch, (1980) (G)
P. radiata	91.7	90 Langer, (1993) (G)
P. contorta	55.0	75 Langer, (1993) (G)
N. solandri	16.3	3-82 Ledgard & Cath, (1983) (E) 65-75 Allen & Platt, (1990) (E,S) 47 Wardle, (1984) (S) 7-57 Wardle, (1970) (S)
N. fusca	38.1	3-78 Ledgard & Cath, (1983) (E) 54 Beggs, (1999) (G) 39 Wardle, (1984) (S)
C. scoparius	61.0	96 Jinadasa, (2000) (G)
S. microphylla	40.0	65 Webb, (1993) (G)
Q. robur	0.0	70 Frost & Rydin, (1997) (G)

Note:

- The method of determining seed viability is indicated in parentheses. G = standard germination trial, S = soundness indicated by the presence of a white endosperm, E = ethanol soak method (Ledgard & Cath, 1983).

The germination of *Nothofagus* seeds in this study lie in the lower range of viability as reported in previous investigations (Table 4.1). This may be a result of the large variability of *Nothofagus* seed quality between sites and years (Wardle, 1970; Ledgard & Cath, 1983) and the differing methods employed to denote seed viability. Commonly, seed viability has been inferred by the presence of a white endosperm and embryo ('soundness') or through the ethanol soak method (Wardle, 1970; Wardle, 1984; Allen & Platt, 1980). The validity of such assumptions is not supported in comparative experiments of these methods (Ledgard & Cath, 1983; Beggs, 1999). Beggs (1999) measured the *N. fusca* viability to diverge between 73% and 58% employing the ethanol soak method and a standardised germination trial respectively. The work of Ledgard and Cath (1983) compared germination, ethanol and soundness tests as indicators of *N. fusca* viability, with the tests estimating 64%, 54% and 53% respectively. These inconsistencies between test methods may further increase the viability disparity between investigations.

Apportioning of mass between the seed coat and embryo-endosperm fractions varied between species. All species pooled apportioned 70% and 30% of the seed mass to the embryo-endosperm and the seed coat fractions respectively. These values exceed the measurements of Grubb and Coomes (1997) on 24 shrub and tree species in caatinga Amazon, where only 17% of the seed mass was apportioned to the seed coat. The difference between studies may

be attributed to the different climatic biomes of the surveyed species and the limited number of species included in the present study. Only eight species were surveyed in this study, of which 3 belonged to the Leguminosae family. This may have inflated the mean mass allocated to the seed coat.

Lee *et al* (1991) found constancy in three Rosaceae species in the seeds' mass allocation between the embryo-endosperm and the seed coat fractions. Similar homogeny was found in the *Pinus* species of the present study, where the allocation of mass varied by 1%. These results suggest possible phylogenetic inertia of this seed trait.

The work of Lee *et al* (1991) on 9 temperate shrub species found 5-89% of the seed mass was allocated to the fibrous testa. This large allocation to the seed coat was predominantly found in invasive species that persist in the soil seed bank. The present study also found evidence for this relationship. The largest mass allocations to the seed coat were found in leguminous *S. microphylla*, *C. scoparius* and *U. europaeus* species, which all persist in the soil seed bank (Zabkiewicz, 1976; Webb, 1993; Baskin & Baskin, 1998; Moles & Drake, 1999).

The higher nitrogen content of the embryo-endosperm fraction is consistent with the localisation of protein reserves in the endosperm (Bewley & Black, 1978). The concentration of nutrients in the endosperm is reflective of the energy and nutrients available for seedling development and exploitative organisms. As shown in Table 4.2, the concentration of nitrogen in the embryo-endosperm and the seed coat fractions in this study was consistent with Lee *et al* (1991) and Grubb and Coomes (1997).

Table 4.2 Nitrogen concentration in the embryo-endosperm and the seed coat fractions.

	Embryo-endosperm ($mg\ g^{-1}$)	Seed coat ($mg\ g^{-1}$)
Present study	9-81	5-25
Lee *et al* (1991)	7-63	2-19
Grubb & Coomes (1997)	9-56	3-16

The water-soluble substances represent the amount of material that may be released by the leaching action of water in the field. The characteristics of the water-soluble component varied between species, with greater differentiation apparent in the ground treatment. The mass of labile extractives in the ground treatment varied between 9% in *N. solandri* to 29% in *U. europaeus*, with the variance principally reflecting the different seed chemistries. The difference in the water-soluble fraction between species is well documented in previous leaf litter studies (Tukey, 1970; Berg & Staaf, 1981; Gunnarsson *et al*, 1988; Vesterdal, 1999). The water-soluble component of fragmented leaf litter (1 cm) of 13 species ranged between 9-28% (Daubenmire & Prusso, 1963). Taylor and Parkinson (1988) found the water-soluble material released from *Pinus contorta* x *P. banksiana* and *Populus tremuloides* comprised 8% and 24% of the foliage mass respectively. The range found in these leaf studies is cognate with the range for ground seeds reported in the present study.

The quantity of water-soluble material increased from whole, to scarified, to ground, resulting in mean mass losses of 0.6%, 4.8% and 16.6% respectively. These results indicate only a minimal release of labile material occurs when the seed coat remains unmodified. The mass of water-soluble substances in the whole seeds ranged between 0-3%. Comparable low values have been reported for whole seeds of *Pisum sativum,* where only 1% of the seed mass was lost after 24 hours leaching (Larson, 1968). The small release of leachates from whole seeds can be ascribed to the leakage of water-soluble compounds as the membranes reconstitute. Seed membranes are fragmented as the seeds are progressively dried during maturation (Simon & Raja Harun, 1972; Short & Lacy, 1976). The hydration of seeds is accompanied by a simultaneous leakage of intra-cellular substances including carbohydrates and amino acids. After a short release period, the integrity of the seed membranes and the impervious nature of the seed coat are restored (Samad & Pearce, 1978).

The protective outer layers of the seeds were breached in the ground and the scarification treatments, increasing the surfaces exposed to water. Removal of these physical barriers increased the mobilisation of the water-soluble fraction. The concentration of reducing sugars, α-amino acid-N and ammonium-N were greatest when extracted from ground seed material. An increased release of organic compounds following the removal of physical barriers was also reported by Larson (1968) for *Pisum sativum* seeds and by Samad and Pearce (1978) for *Archis hypogea* seeds.

The enhanced release of leachates from ground plant tissue has been reported in leaf litter (Gunnarsson *et al*, 1988). The effect of increasing the leaf litter surface area was not as pronounced as it was for seeds. Nykvist (1959) measured the release of water-soluble substances from *Fraxinus excelsior* leaf litter to represent 22% and 29% of the leaf mass in whole and ground treatments respectively. This differential effect of altered surface integrity between the leaf and seed litter is a result of the litters intrinsic substrate properties. The flat shape, comparatively permeable outer cuticle and the fragmented cell membranes (Day, 1983) of the leaf litter would aid in the diffusion of water to all interior leaf cells. The pseudo-spherical shape, semi-permeable outer seed coat (Bewley & Black, 1978) and the membrane repair capacity of seeds (Priestley, 1986; Baskin & Baskin, 1998) restricts the movement of water into the seed, thereby increasing the time required for water to diffuse from the external environment into the internal cells (Simon & Raja Harun, 1972). The effect of fragmentation is more conspicuous in the seed material as a consequence of these effective barriers to water penetration.

In this study the mean labile nitrogen fraction of 25% (range 3-40%) was within the upper limits of leaf litter (<1-25%) (Berg & Staaf, 1981) and lower limits of pollen litter (21-83%) (Greenfield, 1999). This reflects the differential chemistry of these litter components. The leaf material contains low quality nitrogen compounds that primarily function in structure or as waste products imported into the leaf prior to senescence (Kozlowski, 1973; Aerts, 1996). The reproductive material of seed and pollen contain a high protein content. The comparatively lower water-soluble nitrogen component of the seed material may be associated with the high lipid content, imparting hydrophobic properties onto the seed constituents. Additional evidence for this is the nearly significant negative correlation between the seeds labile nitrogen and lipid content ($r=-0.6046$, $P=0.0641$). No significant

relationship between total seed nitrogen and labile nitrogen was found. This is in concert with the lack of correlation found in leaf litter by Berg and Staaf (1981).

The large quantity of soluble nitrogen and minimal mass loss in the whole seed of *C. scoparius* is likely to be the result of experimental error. The seed comprises a thick seed coat, which would be expected to restrict water movement into the seed. The seeds showed no imbibition over the extraction processes, indicating the seed coat integrity was unchanged. The soluble nitrogen from whole seeds exceeded the soluble nitrogen in the scarified and heat-treated seeds by approximately 20%. This value was confirmed by repeated nitrogen (Kjeldahl) determinations (as indicated by the low standard error). In contrast, the concentration of α-amino acid-N and ammonium-N in the whole seed extractive was significantly less than other seed treatments. This indicates the possible contamination of the whole seeds prior to nitrogen determination.

The effect of heat-treatment (autoclave) on seed structure and chemistry was not uniform for all species. Scanning electron micrographs showed the autoclave treatment altered the seed coat in a species-specific manner. The outer layers in *N. solandri* and *N. fusca* appeared increasingly cellular, indicating the removal of surface waxes during seed heating. Fractures in the seed coats of *P. radiata*, *P. contorta*, *T. aestivum* and *H. vugare* were observed. Similar observations were made by Gogue and Emino (1979) in the seed testa of *Albizia julibrissin* following exposure to fire. The leguminous species in the present study showed minimal changes in their surface morphology. Small cracks were present in the surface, however this could not be separated from the possible artefacts of sample preparation in scanning electron microscopy. The legume seeds rapidly imbibed on hydration indicating a change in membrane integrity. This effect of heat, only being apparent following seed hydration, has been recorded elsewhere at lower temperatures. Brits *et al* (1994) found the breakage in the seed testa of *Leucopermum condifolium* was a function of desiccation and not heat at 40°C. Extensive fragmentation of the endotesta was observed on hydration of the seeds, which did not occur if the seeds remained moist throughout the heating process.

The physical alteration of the seed coat via scarification and heat treatment would presumably have comparable effects on the release of labile compounds. The similar mass of water-soluble material in heat and scarified seeds of *U. europaeus*, *C. scoparius, P. radiata*

and *S. microphylla* provide evidence for this (Table 3.8). Conversely, the heat-treated seeds of *N. fusca*, *N. solandri*, *P. contorta* and *H. vulgare* released significantly less material than the scarified seeds. It is feasible that the stringent heat treatment applied caused chemical changes, which altered the protein configuration and thus the protein solubility. This explanation is supported by the qualitative changes in the soluble fractions of nitrogen released. Less α-amino acid-N was present in the leachates from heated *C. scoparius*, *P. contorta*, *N. solandri* and *Q. robur* seeds compared to scarified seeds. This possibly indicates a reduced solubility of protein components due to precipitation. Walker (1994) reported a similar effect on heat-treated plant material. Water-soluble material extracted at high temperatures from ground flowers showed a marked quantitative reduction in comparison to cold water extractions.

The heat treatment (autoclave) employed in this study is unrepresentative of any condition in nature. The water-soluble material was assayed to compliment the decomposition microcosm and *in situ* experiments also included in this study. The selection of such a stringent method of heating was to ensure all seeds were uniformly killed, eliminating any effects of seed respiration on the decomposition experiments. It is possible that similar changes in seed surface morphology and protein configuration occur in the field under less extreme heating conditions. This may alter the composition of the water-soluble substances and thus have implication in the nutrient cycling in ecosystems experiencing high temperatures.

The method used to quantify the water-soluble components of seed material was adapted from Walker (1994). The method of the current study varied with respect to the incubation conditions. To avoid any effects of the solvent on the seed coat integrity and the seed lipid content, low temperature (5°C) instead of the addition of chloroform was used to reduce microbial growth. Microorganisms are capable of growth at 5°C (Floate, 1970; Cairreiro & Koske, 1992). Microbial growth could potentially effect the mass and composition of the water-soluble fraction. The seeds and water extractives showed no visual indication of microbial growth, suggesting 5°C was sufficient to quantify and qualify the seeds' water labile content. The first extract was used for determining the concentration of α-amino acid-N, ammonium-N and reducing sugars. The assumption that the first extraction (following 48 hours leaching) contained the majority of water-soluble substances was based on the work of

Nykvist (1959). This research showed the water-soluble material leached in the first 24 hours of whole *Fraxinus excelsior* leaves contained 71% of the total water-soluble material.

The use of low temperatures may have resulted in an underestimation of the water-soluble component. Nykvist (1959) found the quantity of water-soluble material leached from *F. excelsior,* over a 24 hour period, increased linearly as the temperature was elevated from 3°C to 40°C. The disparate release of water-soluble compounds at different temperatures may instead reflect the different rates of release. Measurements of *Pisum sativum* carbohydrate release at different temperatures (10-30°C) effected the rate of release but not the absolute quantity of carbohydrate after 96 hours (Short & Lacy, 1976). If the influence of temperature is primarilly on the pattern of release, the possible reduction at 5°C would be negligible considering that four extractions were carried out on each sample. The comparable quantities extracted from other plant materials (Nykvist, 1959; Daubenmire & Prusso, 1963; Vesterdal, 1999), suggest that an adequate number of water extractions were performed to remove all accessible labile compounds. Additionally, the effect of moving the material between 5°C and 20°C during the extraction processes may have potentially altered the seeds' membrane integrity, thus possibly inflating the mass of the labile material. The effects of the fluctuating temperature should only be apparent in the measurements of the residual material but not the chemistry from the first extraction.

The results of the present study found the quantity and quality of water-soluble compounds in seed substrates, varied largely between species and with altered seed coat integrity. The absolute quantity of water-soluble material presumably did not vary between whole, scarified and ground treatments. However, the accessibility to the water-soluble component of the seed increased with alterations in the seed coat. The seed coat effectively reduced the movement of material between the internal and external environment. In the natural environment the protected outer surfaces may be breached through physical weathering processes or the comminutive and oxidative action of the biological community (Mason, 1977; Swift *et al,* 1979). In such circumstances, substantial increases in the release of water-soluble compounds would be expected. The accessibility and solubility of the seed compounds has important implications to seed turnover in the field. This is because the water-soluble organic materials represent an easily degraded source of energy and nutrients to the microbial community. In the absence of environmental limitations the litter labile fraction is considered

the key substrate factor governing the early stages of decomposition (Berg & Staaf, 1981). In the case of seed material it is likely the initial stages of decomposition will be principally governed by the physical properties of the seed coat which determines accessibility to the seeds' water-soluble component.

Water-soluble material may contain physiologically active substances that potentially alter the activity of microbes. Seed exudates have shown both inhibitory (Kremer, 1986b) and stimulatory effects on microbes (Harman, 1983; Rolston *et al*, 1991). The results from this study found no inhibitory effect of the seed leachates on soil microbial activity, as all leachates were rapidly colonised. The rapid utilisation of labile organic substrates was signified by the increased pH in leachates (Nykvist, 1959). The presence of fungistatic alkaloidal and cyanogenic glycosidal compounds in the seed coat of legume species is speculated to promote seed longevity in the soil by reducing pathogenicity (Baker, 1989; Halloin, 1983). No evidence of this was found in the legume species surveyed. It is possible that such inhibitory compounds were not water-soluble and therefore not present in the leachates.

A strong correspondence was found between the microbial growth in the leachates and the concentration of carbohydrate and nitrogenous compounds. This suggested higher concentration of labile compounds provided increasingly favourable conditions for microbial growth. The intact seed coat provides an oligotrophic environment in the spermosphere of seeds, thereby reducing the substrates available for microbial colonisation. Halloin (1983) suggested this strategy of defence with regard to *Pisum arvense.* Seeds exhibiting an increased rate of solute release also incurred greater incidence of fungal infection (Short & Lacy, 1976). A comparable response of microbial growth with increased fragmentation of plant material was observed in the leaf litter of *Acer plantanoides* (Gunnarsson *et al*, 1988). Maximum bacterial growth was positively correlated with fragment size and dissolved organic carbon.

Halloin (1983) reviewed numerous studies where the presence of seed phenols, enzyme inhibitors, lectins and lipids have inhibited the growth and activity of microbiota. These studies have little ecological relevance because the assays focused on a limited number of microorganisms and/or employed high concentrations of chemicals, uncharacteristic of the

natural environment. The evidence of inhibition is further compounded by the lack of resolution of causative factors. Without excluding other suppressive effects, the results offer only speculation of the presence of a chemical inhibitor. It is likely that the chemical exudates of seeds in the present study do have a selective effect on the microbial community. Little evidence of altered intra- and inter-species interactions is available in the present study as the net inhibitory action of the leachate was measured. No net inhibition at the artificially high concentrations enlisted in the present study infers little net inhibition is likely in the natural environment. The results presented here suggest there is no net effect of seed chemical exudates on ecosystem processes such as decomposition. This is due to the enzymatic diversity and functional redundancy in the microbial community (Wardle *et al*, 1998).

It should be noted that the substrate quality might have been effected by the collection of seeds directly from the tree or from the litter layer. The sampling techniques may have had subsequent effects on the residual substrate characteristics of the seed, as plant material varies chemically and physically with developmental stage and exposure to decomposition processes. Seeds taken directly from the upper litter layer may have been subjected to various abiotic and biotic decomposition processes depending on the length of exposure (Carlisle *et al*, 1966). Labile compounds can be rapidly lost through leaching or the catabolic activity of soil microorganisms following the release of plant litter (Mason, 1977; Swift *et al*, 1979). This altered substrate quality was demonstrated by the 5 % reduction of water-soluble material in *F. excelsior* leaf litter following 1 week of decomposition (Nykvist, 1959). The decomposition of seeds in the field prior to collection is expected to be minimal due to both the presence of an impervious seed coat and the seed collection period coinciding closely with seed release. The seeds collected directly from the tree may not have been fully developed and therefore lack some of the reserves and properties of naturally dispersed mature seeds. The seeds of *U. europaeus* and *C. scoparius* showed indeterminant flowering patterns. As a consequence, the seeds harvested from the same branch were at varying stages of development, thus affecting the potential seed germination and reserve stores.

The variation in maturity and exposure to decomposition processes has the potential to increase the variability within the seed population. Efforts to reduce the variability within a species were undertaken by sorting the seeds according to coloration as an indicator of seed

maturity and deterioration of the seed. The low variability between replicates, within the seed populations, suggest this was adequate to obtain a reliable estimate of the chemical and physical attributes of the seed populations.

4.3 Seed microbial decomposition in an artificial microcosms environment

The study of seeds in the soil ('seed bank studies') has primarily centred on the longevity of the seeds and the implication of this in agriculture, forestry, regeneration ecology and weed management (Fenner, 1985; Baker, 1989; Gilfedder & Kirkpatrick, 1993; Moles & Drake, 1999). Such seed bank studies have focused on the turnover rate of the seed population as indicated by changes in the population size and modification in the residual seed viability and vigour. Further, some studies have attempted to identify possible factors that influence the turnover rate of the seed population. These factors include the environmental conditions (Abdul-Baki & Anderson, 1972), interactions with organisms (predators, disperser and pathogens) (Weaver & Cavers, 1979; Alvarez-Buylla & Martínez-Ramos, 1990; Kalisz, 1991; Crist & Friese, 1993; Lonsdale, 1993) and the intrinsic properties of the seeds (Thompson *et al*, 1993; Hendry *et al*, 1994). These studies show close parallels to decomposition studies, but vary with respect to the parameters measured as a consequence of the experimental aims. Decomposition studies measure the turnover of a substrate plus the dynamics of its associated nutrients effected by the physico-chemical environment, decomposer organisms, and the substrate quality (Swift *et al*, 1979). The information obtained at this biochemical level can be extrapolated to the population level, to make inferences of the populations' turnover rate. Both areas of research investigated the turnover rate of plant material in the soil system and the associated rate-regulating factors, yet they differ with respect to the level of resolution. Seed bank and decomposition studies are principally focused at the population and biochemical level respectively.

Studies of seed populations in the soil often fail to resolve the fate of the seeds in the subterranean environment. These seeds are simply categorised as 'losses' with no real attempt to identify the mechanisms of seed loss and the fate of the residual seed material. A limited number of studies have provided circumstantial evidence for the microbial fate of seeds by fungal exclusion experiments (Lonsdale, 1993; Jansen & Ison, 1995; Dalling *et al*, 1998;

Leishman *et al*, 2000). These experiments reveal that the susceptibility of seeds to fungal degradation varies between species (Leishman *et al*, 2000) and time of the year (Crist & Friese, 1993). This could be interpreted as reflective of the seed substrate quality and the environmental conditions experienced by the fungal communities.

The current study provides biochemical evidence for the microbial decomposition of seed organic substrates. Seed materials were decomposed in microcosms to allow precise measurements of substrate turnover and nitrogen dynamics under controlled conditions. The use of microcosms allowed the manipulation of single or multiple rate-regulating factors, whilst holding all other factors constant. This process yields tentative quantification and qualification of the manipulated factors' possible roles in the environment. Caution must be exercised in extrapolating the present results into the field situation due to numerous and complex interactions and feedback's between rate-regulating factors (Swift *et al*, 1979).

The microcosm environment is artificial and therefore limited in its ability to model the complexity of the prevailing conditions in the natural environment (Taylor & Parkinson, 1988). The present study used microcosms without a leaching regime. This closed system may have resulted in anaerobic microsites and the accumulation of microbial wastes, both of which have the potential to effect microbial activity (Nykvist, 1959; Williams & Gray, 1974; de Laune *et al*, 1981). The measured decomposition variables of mass loss and nitrogen mineralisation may have underestimated the decomposition of seed substrates. Some of the mineralised nutrients and energy will be incorporated into the microbial biomass potentially inflating residual seed mass and immobilising nitrogen (Swift *et al*, 1979; Nicolartdot *et al*, 1994). Such discrepancies between measured and true decomposition were unable to be accounted for since no measurements of microbial biomass in the microcosms were performed. These potential underestimations are consistent in all microcosm experiments therefore ensuring the validity of comparisons between experiments. Despite the aforementioned limitations the use of microcosms in conjunction with *in situ* experiments assists in understanding the microbial decomposition of seeds.

The results of the present study are discussed in sections utilising the rate-regulating factors categorised by Swift *et al* (1979). These factors are substrate quality, physico-chemical environment and decomposer organisms. These factors act simultaneously to effect microbial

activity and efficiency in decomposing organic substrates. Therefore the method of dividing the discussion up is solely for ease and simplicity of presentation.

Limited former research in seed microbial decomposition restricts comparisons between current and previous data. Extensive research on the decomposition of leaf and wood litters have been carried out. These studies are used for comparative purposes regardless of the intrinsic substrate difference. Such comparisons identify consistent trends between the litter fractions in the decomposition process. It also places seed decay in context with other well-researched litter components.

4.3.1 Substrate quality

The seed substrates generally displayed a curvilinear (Olson, 1963) or linear response (Swift *et al*, 1979) of mass loss over time. The curvilinear response is in accord with the decomposition stages identified by Berg (1986). The initial period of decomposition is dominated by the catabolism of labile compounds and carbohydrates (cellulose and hemicellulose). These high quality compounds are largely concentrated in the embryo-endosperm fraction of the seed. The later stages are dominated by the decomposition of more recalcitrant compounds such as lignin and structural carbohydrates (Berg & Staaf, 1981), which predominate in the seed coat. The different chemistry of the seed embryo-endosperm and the seed coat fractions, govern the initial and final stages of decomposition respectively. A reduced decomposition rate, constant pH and the comparable microbial growth on the seed substrates, were generally observed between 90 and 180 days of incubation. This implied levelling of microbial activity may be attributed to 1) the increased recalcitrance of the substrate, restricting the abundance and diversity of microbes capable of catabolising the residual material (Heal *et al*, 1997), or 2) the occurrence of anaerobic microsites and the concentration of microbial waste products as an artefact of the microcosm design. These two explanations are not mutually exclusive and it is likely that a combination of these is responsible for the reduced rate of decomposition.

Most species exhibit a lag phase. This is evident in the initial stages of mass loss from whole and scarified seeds. The minimal decomposition recorded over this period may reflect a period of microbial adaptation to the new substrate or a period of substrate conditioning. The

adaptation time required for microbes to exploit seed substrates was presumably low, due to the rapid mass loss and nitrogen release from ground substrates in the initial 30 days. It is possible that a lag in the ground substrates would be evident if measurements of decomposition variables were made at a finer time interval. The lag phase decreased with increasing damage to the seed coat, inferring that the lag is primarily associated with seed coat integrity.

Three phases of seed decomposition can be inferred from the lag and curvilinear response of mass loss. The initial lag 'pre-conditioning' phase is dominated by the physical barriers of the seed coat. The structural characteristics of the seed coat are a function of the fraction's lignin and structural carbohydrate content therefore the lag is effectively determined by the chemical properties of the seed coat. The second phase is dominated by the high quality embryo-endosperm fraction and the third phase is predominantly governed by the chemical characters of the seed coat.

Circumstantial evidence exists for the dominance of the seed coat in the later decomposition stages. First, the seed coat contained consistently lower concentrations of nitrogen, indicative of a low quality substrate. Second, the residual material remaining after 180 days of decomposition was largely the seed coat devoid of the embryo-endosperm fraction. Third, there was a close correspondence between the proportional mass of the embryo-endosperm fraction and the plateau of the decomposition rate. This is illustrated in the scarified *T. aestivum*, *U. europaeus* and *C. scoparius* seeds. The characteristic curvilinear mass loss response curbs after 90 days decomposition closely paralleling the mass of the embryo-endosperm fraction as shown in Table 4.3.

Table 4.3 The embryo-endosperm fraction (%) and mass loss (% dry weight basis) after 90 days from scarified seeds (%).

Species	Embryo-endosperm mass (%)	Mass loss from scarified seeds (%) t=90 days
T. aestivum	78	74
U. europaeus	53	44
C. scoparius	58	54

The initial lag phase may not be evident where the seed coat has been altered or only a thin seed coat was present. In the natural environment the seed coat may be altered, in a species-specific manner, through interactions with the abiotic and biotic environment. This includes mechanical abrasion from seed movement in the soil profile, fluctuating environmental conditions (Fenner,1985; Zammit & Zedler, 1994), animal interactions (Copeland & McDonald, 1995) and microbial degradation (van Leeuwen, 1981). These scarifying mechanisms facilitate the access of microbial enzymes, which reduce the lag phase thereby accelerating the rate of seed decay.

The alternating dominance of the seed coat and embryo-endosperm chemistry in the degradation of seed substrates would presumably have a selective effect on the decomposer community. The initial breach of the seed coat, the utilisation of high quality embryo-endosperm and the accumulation of recalcitrant materials would be expected to select for a progressive succession of K-, r- and K-selected microbial species.

The first and third phases of seed decomposition involve the degradation of the seed coat. The recalcitrant materials localised in this fraction would be expected to select for specialist K-selected microbes capable of utilising the refractory forms of energy and nutrients. Taxonomic studies have identified specialised microorganisms on the seed coat providing evidence of these intimate associations (Kremer, 1987). Kirkpatrick and Bazazz (1979) isolated fungi from the seeds of four annuals. Each seed species exhibited specialised fungal associations exhibiting differential efficiencies at penetrating the seed coat. Conversely, the work of Crist and Friese (1993) in the shrub-steppe ecosystem found that the fungi present on the seed coat were predominantly cosmopolitan soil species. These disparate studies suggest the importance of specialised microbial association to breach the seed coat barrier may be species-specific, possibly reflecting the design of the seed coat.

The second phase is associated with the metabolism of the high quality embryo-endosperm. The chemical characteristics of this fraction would presumably select for resource non-specific microbes predominantly exhibiting r-selected life histories. The breach of the seed coat would be followed by a flush of microbial growth in response to the increased substrate availability. This would presumably result in a highly competitive microbial environment due to the large diversity of microbes capable of metabolising the water-soluble nitrogenous compounds, sugars and organic acids. The microbes that are capable of decomposing the seed coat fraction may be displaced or alternatively these microbes may also metabolise embryo-endosperm compounds. Microbial species recognised as resource specific, such as Basidiomycetes, are facultative with regard to the decomposition of low molecular weight compounds (Hammel. 1997). In the presence of polysaccharides, the fungi preferentially degrade these, as less energy is invested in the production of enzymes and assimilation of the associated energy and nutrients.

The resolution of these three phases is only speculation. Understanding the role of the seed fractions on the pattern of decomposition would require extensive research on the degradation of the seed components in isolation and taxonomic studies to identify the succession of microorganisms. The generality of such divisions in the decomposition process will be effected by the large intra- and inter-species variations in seed physical and chemical properties plus the numerous feedback's of substrates quality as decomposition progresses. Despite the inherent complexity of the decomposition process, this division into three phases offers a simplified framework to explain the progression of seed degradation.

The whole seed material was rapidly decomposed in microcosms as indicated by the mean mass loss and net nitrogen mineralisation measured as 35% (range 0-78%) and 30% (range 0-70%) respectively, after 180 days incubation. Despite the different test species surveyed, the measured 11% (range 0-40%) mass loss after 30 days, confirms the 15% (range 0-35%) mass loss from seeds measured by Greenfield (1998). The rapid turnover rate is consistent with other reproductive litters with similar high quality substrates. Ground flower litter lost 40-70% mass after 180 days incubation *in situ* (Walker, 1994). A survey of pollens from 35 species found 27-62% mass loss and 5-67% net nitrogen mineralisation after 30 days decomposition in microcosms (Greenfield, 1999). The higher values of decomposition

variables obtained from these studies is likely to be a reflection of the small particle size of both the ground flower and pollen litters, thereby increasing the surface area exposed to microbial enzymes. Comparable values to the aforementioned studies were obtained from ground seed material in the present study. These values ranged between 10-50% mass loss and 5-42% net nitrogen release in the first 30 days of decomposition.

The influence of whole, scarified and ground treatments on the seed mass loss was most pronounced in the initial 30 days of decomposition. Mass loss was predominantly greatest in the ground seed treatment. This increased decomposition for ground seed material is associated with the removal of physical barriers imposed by the seed testa to microbial exploitation and the increased embryo-endospermic surfaces available for microbial colonisation. Greenfield (1998) found comparable increases in mass loss between whole and ground seed material in the initial 30 days of decomposition. The increased rate of decomposition associated with increased fragmentation of surfaces has also been observed in other litter fractions. Boddy (1983) measured a 40% increase in the rate of carbon dioxide evolution when *Fagus sylvatica* branch litter was reduced from 10 cm^2 to 5 cm^2 samples.

The difference between treatments becomes less marked over time. The calculated mean decay constant from whole, scarified, heat and ground seeds equalled 1.01, 1.47, 1.21, and 1.48 respectively. This increasing homogeny between the treatments suggests sufficient time had lapsed to degrade the seed coat and to access the nutritional embryo-endosperm fraction in each treatment. The whole seeds of *S. microphylla* and *C. scoparius* are exceptions. This infers that a greater timeframe was required to overcome structural and possibly chemical barriers to the exploitation of the embryo-endosperm fraction. Comparable homogeny between whole and ground treatments over time was found in the leaf litter of *Fagus sylvatica* (Scheu & Wolters, 1991). The carbon mineralised from ground (<1mm) leaf litter exceeded the corresponding release from whole leaf material in the initial phases of decomposition. After 161 days, the carbon mineralised from whole and ground material was comparable.

With the exception of *Q.* robur, net nitrogen mineralisation occurred in all microcosms. These results conform to the generalisation that net nitrogen release occurs when the initial nitrogen content is greater than 1.7-2.0% (Palm & Rowland, 1997). The nitrogen release

from seeds was not always concomitant with mass loss. For example, *P. radiata*, *T. aestivum* and *N. fusca* seeds incurred the greatest mass loss after 30 days from ground seed material. However, the nitrogen release was similar or lower than the other treatments involving altered seed coat integrity. Such discrepancies between these decomposition variables are related to the opposing processes of nitrogen mineralisation and immobilisation (Heal *et al*, 1982). The ground material increases the colonisable surfaces and access to energy rich resources thereby supporting comparatively greater microbial populations. As a consequence, the microbial community is nitrogen limited thus resulting in the immobilisation of nitrogen into the microbial protoplasm.

The mass loss and nitrogen mineralised from whole seeds varied significantly between species, reflective of the divergent chemical and structural characteristics of each species. The large variation between species is consistent with decomposition studies of leaf litter (Gosz *et al*, 1972; Howard & Howard, 1980). The influence of seed physical and chemical attributes is illustrated in the whole seeds of *S. microphyla* and *Q. robur* respectively.

The large water-soluble fraction, the high nitrogen concentration and the low C:N ratio of *S. microphlla* seeds infers a high quality substrate. The seed chemistry predicts that the microbial community should rapidly exploit the associated energy and nutrients. Conversely, no mass loss or net nitrogen mineralisation was recorded from whole seeds after 180 days incubation. Additionally, no morphological changes in the seeds, indicative of degradation were evident over the entire experimental period. The inability of microbes to exploit the high quality substrates associated with *S. microphylla*, is attributed to the hard seed coat. The seeds outer multi-layered membrane effectively excludes the movement of moisture and microbial enzymes to the high quality embryo-endosperm fraction. In seed treatments where the seed coat integrity is altered the seed substrates were rapidly metabolised. In heat and ground treated seeds, 50% of the seed mass loss was measured in the initial 30 days of decomposition.

The effect of differential seed chemistry on nitrogen release is illustrated in *Q. robur* seeds. The seeds of *Q. robur* experience extensive mass loss indicative of microbial metabolism, however only a negligible amount of mineral nitrogen was detected in the microcosms. This is attributed to the high proportion of carbonaceous material, denoted by the high C:N ratio

of 61. The carbon material provided energy for heterotrophic microbial metabolism and tissue synthesis. The nitrogen mineralised through the oxidation of the energy-rich substrate is assimilated into microbial protoplasm and immobilised. As the seed is progressively depleted of carbon sources a critical limit is met where net nitrogen mineralisation occurs, since nitrogen is no longer limiting to the microbial community. This critical C:N ratio is estimated to be between 20-30 corresponding to 1.7-2.5% nitrogen concentration (Berg & Staaf, 1981; Heal *et al*, 1982). No net nitrogen release was measured over the 6 month experimental period. It is possible that the critical limit was reached in this study however the nitrogen mineralised may have been complexed by tannins (Steele *et al*, 1993) further reducing the rate of nitrogen release.

The water-soluble chemical analysis revealed *Q. robur* seeds contained large concentrations of reducing sugars (14 mg g^{-1}). The release of this readily degradable energy source would be expected to stimulate mycelial production. This would increase the utilisation of mineral nitrogen thereby immobilising nitrogen. A similar effect was observed when leaf litters were supplemented with glucose. Jones and Richards (1978) measured a significant reduction in the quantity of exchangeable ammonium-N in the presence of glucose. This effect was attributed to the nitrogen being assimilated into fungal protoplasm.

The differential decomposition rate of species, resulting from the species chemical and physical attributes, is in accord with the divergent seed survivorship observed in seed bank studies. The chemical exclusion of fungi from seeds in the soil environment has shown to either enhance (Lonsdale, 1993; Dalling *et al*, 1998; Leishman *et al*, 2000) or have only a minimal effect (Jansen & Ison, 1995) on seed longevity. The presence of fungicides reduced annual seed losses of *Miconia argentea* and *Cecropia insignis* by 39% and 47%, respectively (Dalling *et al*, 1998). Additionally, a location effect was observed in fungicide treated seeds. In the *Miconia argentea* seeds, the fungicides had a decreased effect on seed survivorship relative to the distance from the parent canopy. This may imply a reduced pressure from fungal decomposers. Leishman *et al* (2000) reported the presence of fungicides increased the survivorship of *Rubus fruticosus* and *Medicago lupulina* by 15% and 28% respectively following burial in the soil environment for two years. No effect of fungicide was measured in the other two species surveyed (*Convolvulus arvensis* and *Lotus corniculatus*). This further illustrated the species-specific effect of fungi on seed survival.

Heat-treated seeds showed a varied response between species. As mentioned earlier in the discussion on water-soluble substances, the effect of heat treatment was species-specific. The heated seeds contained altered seed surfaces as a consequence of the removal of surface waxes or fractures in the seed coat. Presumably this enhanced the accessibility of the embryo-endospermic seed substrates to microbes. The mass losses incurred in *T. aestivum*, *H. vulgare*, *P. radiata*, *N. solandri* and *Q. robur* did not support this (Table 3.12). The heated seeds consistently showed lower mass losses over all incubation periods compared to other whole, scarified and ground treatments. This may be accounted for by qualitative changes in seed chemistry thus increasing the recalcitrance of the seed substrate. A comparable pattern of decomposition was found with autoclaved seeds *in situ*. Autoclave treatments were used to provide a uniform method to kill all seed species and to avoid any confounding effects of seed respiration on the mass loss experiments. The analysis of the water-soluble substances indicated changes in the substrate quality that may influence the rate of decay. These qualitative changes reduced the value of the heat-treated seeds as a control to remove any artefacts of seed respiration. The results of the heat-treated seeds will not be discussed further. The artificial conditions that the seeds were subjected to have little relevance to the conditions in the natural environment.

Multi-species comparisons of litters have attempted to identify indices applicable to a range of litter components and sites. The nitrogen (Staaf & Berg, 1982), lignin (Berg, 1986), labile extractives (Nykvist, 1959), carbon : nutrient ratio (Witkamp, 1966; Taylor *et al*, 1989a; Hector *et al*, 2000) and lignin : nutrient ratio (Meentemeyer, 1978; Melillo *et al*, 1982; Keenan *et al*, 1996) values of leaf litters have been advanced by numerous studies as good predictors of mass loss. These indices appear to have limited applicability to whole seed material. It is likely that the protective seed coat masked any predictive value of seed chemical composition. No elemental component had a strong ability to account for the inter-species differences in seed decay. Seed nitrogen content showed a correlation with nitrogen release after 180 days incubation ($r=0.4262$, $P=0.0188$), but this was considered too week to warrant further discussion. This disparity between leaf and seed material is not unexpected, due to the intrinsic differences in their physical and chemical attributes.

Recently, numerous researchers have postulated the superior predictive power of physical litter attributes in forecasting the rate of decomposition (Gallardo & Merino, 1993; Gillon *et al*, 1994; Cornelissen & Thompson, 1997; Cornelissen *et al*, 1999). This was well illustrated in leaf litter studies of Mediterranean ecosystems. The leaf material generally possessed thick protective surfaces as an adaptation to the hydrological and edaphic constraints of the climate. The relatively high lignin and cutin in the leaf impart properties of impermeability to water and impenetrability to fungal hyphae. Collectively this is measured as leaf 'toughness'. This index of leaf 'toughness' proved an important predictor of the leaf decomposition rate in these systems (Gallardo & Merino, 1993; Gillon *et al*, 1994). The impermeable nature of the seed coat displayed close functional parallels to the leaf material of Mediterranean ecosystems, suggesting the physical, rather than chemical properties of the seeds may have better predictive value.

It was proposed by Cornelissen *et al* (1999) that the protective traits of leaves and their effect on the consumers continue into the soil. The functioning of such traits have the potential to alter the decomposition rate. This statement is highly applicable to seeds, as defensive traits are operational following release from the tree. The correlation analysis of the seeds' physical and chemical parameters with whole seed decay constants revealed that the distribution of seed mass between the embryo-endosperm and seed coat fractions explained the greatest degree of inter-species variation in the decomposition rate. The rate of decomposition of whole seeds was inversely correlated with the allocation of seed mass to the seed coat fraction ($r=-0.7058$, $P=0.0001$). This indicates that seeds with a thick intact coat are less susceptible to microbial degradation. This further reiterates the importance of the seed coat in determining the rate of seed decomposition. It should be noted that the predictive power of this correlate is limited to the ten test species decomposed under the selected controlled conditions of the present study (Daubenmire & Prusso, 1963).

The physical barriers of seeds are a function of the fraction's chemical attributes (Palm & Rowland, 1997). More extensive chemical analysis of the seed fractions may provide an insight into the inhibitory chemical and physical mechanisms to microbial decomposition. The water-soluble assays suggested no net inhibitory action by labile compounds on microbial activity. Regardless, it is feasible inhibitory compounds are present in a non-labile form. In a survey of 80 temperate species, Hendry *et al* (1994) found a significant positive

correlation between seed persistence in the soil and the concentration of *ortho*-dihydroxyphenol. The presence of *ortho*-dihydroxyphenol was proposed to be inhibitory to microbes thus decreasing the seed's susceptibility to microbial decay.

A significant positive correlation between seed viability and mass loss from whole seeds was found in the ten test species. This does not support the general notion that seeds with a high viability are less susceptible to microbial attack. Three explanations for this relationship are suggested. First, the germinating seedlings would result in mass loss via seedling respiration. This explanation is considered unlikely because all germinating seedling were promptly harvested on the appearance of the radicle to arrest further seedling growth. Additionally, the enhanced mass loss effect would be expected to be most evident in the early stages of decomposition when the majority of germination occurred. However, no significant correlation between viability and mass loss was found in the initial 30 days of decomposition. Second, during the germination process the seed mobilises high molecular weight compounds by enzymatic degradation of storage tissue to provide energy and nutrients to the axis of the developing embryo (Bewley & Black, 1978; Bryant, 1985). This may also enhance the substrate quality of the seed by increasing the proportion of low molecular weight compounds present. Third, the germination of seeds and the harvesting regime used in this study, provided entrance sites to opportunistic microorganisms. The physical barrier of the seed coat was effectively removed by the protrusion and removal of the seed radicle. These latter two explanations appear more likely since microbial growth was extensive following the detachment of radicles in *P. radiata*, *U. europaeus*, *T. aestivum* and *H. vulgare* species, indicative of an increase in substrate availability (Nicolardot *et al*, 1994). The equivalent in the field of such seedling harvesting may occur through the grazing action by animals (Downey & Smith, 2000) and extreme environmental conditions (Wardle, 1970). These results indicate that the germination of seedlings and their exposure to adverse conditions may increase the mobilisation rate of seed nutrients in the field.

Immature seeds provide a mechanism where the seed coat integrity is not fully established. The premature membranes impart properties of enhanced seed permeability to water and microbial enzymes. Poorly developed seeds are often susceptible to fungal attack under moist conditions (Webb, 1993; Copeland & McDonald, 1995; Baskin & Baskin, 1998). The rapid imbibition of water by immature seeds of *U. europaeus*, indicated an increased membrane

permeability. These seeds contained a reduced nitrogen concentration compared to mature seeds. The lower nitrogen content is possibly due to the incomplete filling of seeds with storage provisions prior to its harvesting. Despite this reduction, the nitrogen comprised 4.8% of the seed mass, inferring a high quality substrate (Swift *et al*, 1979). The nitrogen of immature *U. europaeus* seeds was rapidly mobilised by the microbial community. Over 60% of the seed nitrogen was released during the 180 days of decomposition. During the initial 30 days, decomposition proceeded more rapidly in comparison to mature seeds. This increased rate in the initial stages of decomposition is presumably a result of the increased permeability of the seed coat. This difference between the immature and mature seeds was not significant after 90 days incubation. The mature seeds had undergone sufficient substrate conditioning of the seed coat to allow access by the microbial enzymes to the embryo-endosperm fraction.

The feeding activity of insects on seeds provides another natural mechanism to overcome the physical barrier of the hard seed coat (Janzen, 1977; Mills, 1983). The subsequent damage to the seed coat provides an entrance site for soil- or seed-borne microbiota to exploit the highly nutritional seed embryo-endosperm tissue. The seed coat of *S. microphylla* and *U. europaeus* were ruptured prior to seed maturation, by the feeding activities of *Stathmopoda aposema* and *Apion ulicis* larvae respectively (Hill *et al*, 1991; Webb, 1993; Sullivan *et al*, 1995). Microscopic examinations of the insect damaged seeds revealed a concentration of fungal mycelia at the sites of seed coat rupture. Kremer and Spencer (1989) observed a similar intimate association between insect puncture wounds and fungi in *Abution theophasti* seeds.

Insect feeding activity may alter the quality and viability of the seeds (Mills, 1983). The feeding activity of *S. aposema* larvae was reported by Webb (1993) as being lethal due to its depletion of the *S. microphylla* seed's embryo-endosperm fraction (Webb, 1993). Consequently, the residual seed material should be dominated by the seed coat chemistry. The measured nitrogen content of the *S. microphylla* seed coat (9 mg g^{-1}) and the insect damaged seeds (26.8 mg g^{-1}) does not support this. The elevated nitrogen content of insect damaged seeds may be a result of the incomplete removal of the embryo-endosperm fraction, the deposition of insect frass, and the presence of microbial tissue. The nitrogen retained in the seed is of seed origin, however it is being transferred, in insect frass and microbial tissue, to the decomposer community as a secondary substrate.

The elevated nutritional value of insect-damaged *S. microphylla* seeds may be partly explained by the seed collection method used. These insect damaged seeds were taken directly from the tree, prior to seed fall. Live larvae were removed during seed extraction to arrest further depletion of the seed reserves. This may have resulted in the retention of emryo-endoperm material in the seed coat cavity. This is further supported by the observed germination capacity exhibited in a selected number of insect-damaged seeds.

The collective primary and secondary substrates of these insect damaged seeds were readily colonised and mobilised by microorganisms in *U. europaeus* and *S. microphylla* species. The rapid exploitation of insect damaged seeds has important implications for seed turnover in the field especially in *S. microphylla*. The other simulated natural scarification mechanisms in this study, such as fluctuating temperature and moisture regimes, had little effect on the seed coat integrity. Where the seed coat remained intact no mass loss or net nitrogen mineralisation was measured. The consumption and egestion of plant material rendered it more accessible to the microbial community with the mass loss closely paralleling the losses incurred from ground seed materials. These results suggest that in the natural environment insects play an important role in the turnover of *S. microphylla* seeds. The insect damaged seeds represent 11-28% of the total annual seed production (Hill *et al*, 1991; Webb, 1993). The measured decay rate in the present study predicts that a 95% turnover of this fraction will occurs in under 1.2 years.

The nitrogen released from insect damaged *S. microphylla* seeds was 30% less than the corresponding ground seeds after 180 days incubation. The reduced mineralisation may be related to the increased recalcitrance of nitrogen forms following processing by insect larvae. Evidence for this is provided in the comparatively reduced labile α-amino acid-N component in the insect damaged seed water extractives (Table 3.8). Floate (1970) observed a similar reduction in nitrogen release from secondary substrates. The decomposition of *Nardus* and *Agrostis-Festuca* following sheep digestion and excretion was significantly lower than the decomposition of the corresponding primary substrates. The release of mineral nitrogen was halved after animal processing. The comparatively reduced effect of insect consumption on the nitrogen release observed in the present study, is attributed to insect frass being composed largely of undigested plant tissue (Gosz *et al*, 1972).

Kremer and Spencer (1989) proposed the use of insects and the ensuing soil microbial degradation as a control agent for invasive weed species. The feeding action of the *Niesthrea lousianica* insect increased the incidence of fungal infection significantly and reduced the seed viability of *Abutilon theophrasti*. The work of Kremer and Spencer (1989) did not qualify the effect of insects and microbes on plant annual recruitment and therefore its capacity to control plant populations. The increased microbial exploitation of insect-damaged seeds is supported by the data in the present study for *S. microphylla* and *U. europaeus*. However, the role of insect-microbe interaction in reducing plant species fitness is questionable. The impact of *Apion ulicis* larvae on *U. europaeus* seed recruitment is limited due to its reproductive asynchrony with seeding events. Hill *et al* (1991) estimated 60-70% of the annual seed production is infested with larvae, however this was insufficient to reduce plant fitness. The effectiveness of a control agent is dependent on its ability to eliminate annual recruitment to a quantity below that required to maintain the existing population. Further, the impact of insects and microbes on the plant will depend on the timing of the loss, the environmental conditions, compensatory mechanisms such as re-growth and the frequency of favourable microsites for successful seedling establishment (Crawley, 1992; Downey & Smith, 2000). It is unlikely that the collective action of both insects and decomposer microbes will control the spread of *U. europaeus*.

The concentration of seed units within the microcosm is not characteristic of the distribution of seeds in the field. The hydration of seeds results in the release of high quality organic compounds, as the membranes reconstitute. The presence of these labile compounds may expose the seeds to atypically high microbial pressure (Hayman, 1969; Short & Lacy, 1976; Lynch, 1978) and/or produce a 'priming effect' on the microbial population for the degradation of the resistant seed coat (Taylor & Parkinson, 1988). The removal of the labile material would be expected to reduce microbial activity. No evidence of this however was found on visual observations of the microcosms. The removal of labile compounds resulted in a 5% increase in net nitrogen mineralisation during the initial 30 days of incubation. The effect of labile extraction was negligible after 90 days. The increased release of nitrogen in the initial stages of decomposition can be explained by the residual seed chemistry following water extraction. The water-soluble leachates are predominantly carbonaceous. The whole seeds mobilised 0.0-4.6 mg g^{-1} of reducing sugars whereas the combined quantities of ammonium-N and α-amino acid nitrogen ranged from 0.00-0.19 mg g^{-1}. It is recognised that

these values do not include all the nitrogenous or carbonaceous material in the leachates, but they may provide an insight into the proportions of these nutrients. The increased proportional reduction of carbon via the removal of labile compounds resulted in a carbon limitation to the microbial community. Consequently, the nitrogen excess to microbial requirements was released into the surrounding medium. This resulted in an accumulation of mineral nitrogen ions. Ideally, this experiment should have been complimented with mass loss measurements, but due to equipment restraints this was not feasible. A similar increase in net nitrogen mineralisation after the removal of labile compounds was reported by Handayanto *et al* (1997). The green labile-free leaf material of *Calliandra calothyrsus* displayed an increased release of nitrogen. This was attributed to a reduction in the water-soluble carbon and polyphenols. The removal of these compounds reduced the immobilisation of nitrogen in microbial protoplasm and the complexing of nitrogen with polyphenolic compounds.

The rapid decomposition of ground seed material and the mobilisation of its associated nutrients, exceeded the values obtained for leaf and twig material (Table 3.30). This trend of increased decomposition from wood, to leaf, to reproductive litter is consistent with that reported by Swift *et al* (1979). The differential decomposition rate can be attributed to the consistently higher nitrogen concentrations and decreased structural compounds in seed substrates thereby constituting a comparatively higher quality substrate.

In the field, plant litter components rarely decompose in isolation. The plant litter is composed of a consort of plant tissue displaying characteristic substrate qualities and consequent decomposition rate. The intimate association between intra- and inter-species litter components is further complicated by the non-additive interactions and numerous feedback's between litters when decomposed in mixtures. This study, in accord with numerous inter-species litter mixture research, found a combination of positive (Taylor *et al*, 1989b; Klemmedson, 1992; Wardle *et al*, 1997; Chadwick *et al*, 1998; Blair *et al*, 1990), negative (Fyles & Fyles, 1993) and neutral (Blair *et al*, 1990; Briones & Ineson, 1996; Scowcroft, 1997) interactions by combining litters of different substrate qualities. The combination of *U. europaeus* seed and branch material displayed a synergistic interaction, resulting in greater mass loss and nitrogen release during decomposition than predicted by summing their individual parts. This is in accord with Wardle *et al* (1997), who observed the

mixing of high quality litters was more likely to enhance the decomposition of its components. Previous studies have prescribed the positive interactions, as decomposition proceeds, to the increased nutrient homogeny of the litter components. The limiting nutrients move from high to low quality litter by water film diffusion (Chadwick *et al*, 1998) or fungal translocation (Taylor *et al*, 1989b; McTiernan *et al*, 1997). Additionally, the composite of litters may have micro-environmental effects resulting in changes in the physical environment or the decomposer community (Blair *et al*, 1990; Hector *et al*, 2000). The use of ground material in the present study, restricts the analysis to net changes in the collective litters. Consequently, no residual analysis was performed on the individual litter fraction to identify any movement of nutrients between the litter components. Identifying the mechanisms responsible for these interactive effects would require further research.

Conversely, the combination of seed and needle litter of *P. radiata* greatly retarded the mass loss and net nitrogen mineralisation. The reduced rate of decomposition extended the time required for a 95% mass loss from 3.2 years to 10.0 years in pure seed and seed mixtures respectively. The antagonistic effects may be attributed to phenolics and tannins in the needle litter. The inhibitory action of phenols on fungal activity has been reported *in vitro* (Harrison, 1971; Polglase *et al*, 1992). It is likely similar negative effects will apply in the natural environment. Furthermore, the phenolic compounds may complex with seed proteins increasing the recalcitrance of the seeds nitrogenous compounds (Northup *et al*, 1995). The mixing of such high and low quality litters infers a cost with regard to the rapid recycling of seed nitrogen. However, the immobilisation benefits the systems long-term productivity by reducing the loss of nitrogen (Handayanto *et al*, 1997). Seed release generally occur in the spring and summer months. The present study indicates seed nutrients, decomposed in isolation, are rapidly mobilised within 6 months. This pattern of release coincides with the maximum period of leaching from winter rains. Therefore, the presence of these more recalcitrant materials of other litter components complexes nitrogen and prevents nitrogen losses from the soil system.

The *Nothofagus* litters displayed positive, negative and nil effects of litter mixtures depending on the litter fraction combination. When seed, leaf and twig litters were decomposed in combination, the mass loss was the same or elevated from that predicted. In both cases the nitrogen release was less than predicted. This infers that the heterogeneity of

substrates of the litters stimulated microbial activity. The diversity of the substrates provides a compliment of compounds that aid in the degradation of the more refractory material concentrated in the leaf and twig litters. The elevated microbial activity results in a microbial nitrogen limitation and its concomitant immobilisation. Comparable immobilisation of nitrogen has been observed in other studies (Klemmedson, 1992, Fyles & Fyles, 1993). A similar response was reported in the decomposition of *Eucalyptus globulus* and *Quercus petraea* leaf litters in combination (Briones & Ineson, 1996). The increased rate of carbon dioxide evolution and the retention of mineral nitrogen was attributed to the increased growth of microbes on *Q. petraea*.

The time course of decomposition is important in determining a significant 'mixture effect'. For example, the litter mixtures of *P. radiata* closely paralleled the predicted mass loss in the initial 30 days suggesting no interaction between seed and needle litters. However, the actual and predicted mass loss diverged significantly after 90 days incubation. Briones and Ineson (1996) also observed comparable interactions of litter mixtures over time. The present results indicate the 'mixture effect' will be dependent on both the chemical constituents of the litters and the progression of the decomposition process.

The net decomposition variables measured indicate that the decomposition of litter mixtures is not readily predictable from the decomposition of its individual components in isolation. The unpredictable non-additive effect of intra-species mixtures is consistent with inter-species assemblages (Wardle *et al*, 1997). The presence of multiple litter components is likely to complicate the mobilisation of seed nutrients in the natural environment.

4.3.2 Physico-chemical environment

The mean decomposition of all seeds were slightly greater at a constant (20°C) rather than a fluctuating (12 hours 5°C - 12 hours 15°C) temperature regime. The mean decay rate increased from 0.88 under fluctuating temperatures to 1.09 under constant temperatures. The mean nitrogen release showed an inverse response. A greater proportion of the seed nitrogen was released under fluctuating temperatures. An increased decomposition with temperature elevation, within the limits of mesophilic microorganisms, has often been reported in leaf litter experiments (Witkamp, 1966; Anderson, 1973; Floate, 1970; Boddy, 1983; Clein & Schimel, 1995; Hobbie, 1996). The effect of fluctuating temperatures (18 hours 4°C - 6 hours 10°C) on lichens *Pseudocyphellaria colensoi* and *P. dissimilis* displayed a similar reduction in mass loss when compared to constant temperatures (15°C) (Greenfield, 1993). These results from lichens are consistent with the present seed substrate findings.

The reduced mass loss and increased net nitrogen mineralisation can be attributed to the influence of temperature on microbial activity and their associated enzyme kinetics. The constant conditions are more conducive to microbial growth, resulting in an enhanced turnover of seed substrate and immobilisation of nitrogen. The lower temperatures used in the fluctuating regime are likely to alter the microbial composition (Daubenmire & Prusso, 1963) and possibly cause the partial inhibition of microbial enzymes (Nicolardot *et al*, 1994). The resultant decline in microbial growth and degradation efficiency is associated with the reduced accumulation of nitrogen in microbial protoplasm.

The reduced microbial activity may be counteracted by the effect of the fluctuating temperatures on the seed substrate quality. Diurnal fluctuations in temperature have been proposed as a possible mechanism in seed softening. The oscillating temperatures may cause expansion and contraction of cells eventually disrupting the seed's testa. This may explain the non-significant effect of the temperature regime observed in *U. europaeus*. Seed softening reduced the physical barriers to microbial degradation of the embryo-endosperm. This may have negated the reduced mass loss due to the reduced microbial activity.

The effect of the temperature regime on mass loss varied between species with a 1% difference measured for *T. aestivum* to a 17% difference for *H. vulgare*. The differences may be related to the diversity energy and nutrient forms found in the seed substrates. Nicolardot *et al* (1994) found the influence of temperature on decomposition was related to the complexity of the substrate. Over 140 days, temperature elevations from 4°C to 12°C increased carbon mineralisation from 41% to 48% in glucose and 34% to 53% in the more complex holocellulose substrate. The differential effect of the temperature regime on seed decomposition may be related to the varying complexity of the seed substrates. A more detailed analysis of the chemical constituent of the seeds is required to substantiate this.

The exposure of seeds to periodic reductions in moisture and temperature resulted in a reduced decomposition rate. With the exclusion of *S. microphylla* (due to the negligible mass losses measured in all treatments), the mean time required for 95% mass loss increased from 3.6 years under constant conditions to 4.5 years under repetitive wet-and-dry cycles and 9.0 years under repetitive freeze-and-thaw cycles. This reduction is presumably a result of the decline or cessation of microbial activity under these unfavourable circumstances. The stress conditions select for species able to persist via a reduction in metabolic activity or the formation of resting structures (Van Gestel *et al*, 1993). On the restoration of favourable conditions, the surviving microbes rapidly respond and exploit the substrates in the less competitive environment. Numerous soil studies have measured a rapid increase in the rate of carbon dioxide evolution following temperature or moisture stress (Orchard & Cook, 1983; Bottner, 1985; Van Gestel *et al*, 1993; Schimel & Clein, 1996). This increase in soil respiration indicates the rapidity of the microbial response to the restoration of favourable conditions. The characteristic flushes of carbon dioxide evolution following desiccation or freeze events have been attributed to the residual microbial populations utilising the lysed microbial cells and the physically altered soil organic matter.

Assuming the reduction in mass loss from seeds exposed to wet-and-dry cycles and freeze-and-thaw cycles were principally a reflection of reduced microbial activity during unfavourable conditions, the mass loss under cyclical conditions should approximate the mass loss incurred when seeds are exposed to constant conditions for half that time. Presumably the seed substrates and associated decomposer microorganisms would be exposed to constant conditions of moisture and temperature for effectively the same length of

time. As shown in Table 4.4, this is supported in only a few cases. This implies that the mass losses were a cumulation of the more complicated interactions of episodic stresses on the microbial community and the substrate quality.

Table 4.4 Mass loss (%) from seeds exposed to constant conditions for 90 days and fluctuating temperatures and moisture for 180 days.

Species	Constant 90 days	F+T 180 days	W+D 180 days
S. microphylla	0.0	0.4 (+)	0.4 (+)
C. scoparius	8.7	14.6 (+)	10.7 (0)
U. europaeus	19.0	19.6 (0)	37.2 (+)
P. radiata	27.2	19.4 (0)	25.1 (0)
P. contorta	19.1	13.4 (-)	14.9 (0)
N. solandri	36.7	18.1 (-)	39.5 (0)
N. fusca	38.4	10.9 (-)	35.2 (0)

Note:

- Figures assimilated from Tables 3.26 and Table 3.28.
- F+T = Freeze-and-thaw cycle, W+D = wet-and-dry cycles.
- The symbols in parentheses indicate the differences determined by the Least Significant Difference multiple mean comparison at $\alpha = 0.05$. + = value is significantly higher, - = value is significantly lower, 0 = values are homogenous.

The comparatively reduced mass losses measured in *N. solandri*, *N. fusca* and *P. contorta* seeds exposed to repetitive freeze-and-thaw cycles can be attributed to the selective effect of such perturbations on the microbial community. Taxonomic studies on temperate and Arctic soils have indicated a decline in microbial diversity with reductions in temperature (Latter & Heal, 1971; Carreiro & Koske, 1992). The physiological stresses imposed by repetitive freezing events have been shown to successively reduce the abundance of microbes and hence their efficiency to decompose soil organic matter (Schimel & Clein, 1996). A single freeze-and-thaw event can kill up to 50% of the viable microbial population (Skogland *et al*, 1988; De Luca *et al*, 1992). The effect on soil microbes is dependent on the periodicity, severity and length of the freeze event. De Luca *et al* (1992) reported that freeze events of less than -15°C were required to rupture most microbial cells. The comparatively mild temperature (-4°C) used in the present study probably only had a moderate effect on the microbial community.

The seeds of *S. microphylla*, *P. radiata*, *U. europaeus* and *C. scoparius* displayed either no effect or increased mass loss under freeze-and-thaw conditions (Table 4.4). The increased

mass loss may be associated with the ability of microbes to respire at sub-zero temperatures and the altered physical properties of the substrate. It is often presupposed that microbial respiration is arrested at sub-zero temperatures, due to the absence of free water and the kinetics of microbial enzymes. This assumption is invalid, as free water and microbial respiration have been measured at -5°C. While the rate of respiration is markedly reduced, over time its cumulative effect may substantiate a significant amount of substrate decomposition. Numerous studies in temperate and Arctic communities have indicated that the respiration of microbes at lower temperatures accounts for between 25-60% of the annual carbon mineralisation (Coxon & Parkinson, 1987; Taylor & Jones, 1990; Sommerfeld *et al*, 1993). These high figures are a consequence of the long winters at low temperatures and the short summers being relatively non-conducive for high microbial activity. However, they serve to reiterate the continued activity of microbial communities at sub-zero temperatures.

The repetitive fluctuations in environmental conditions also represent a perturbation to the litter substrates. Ice crystals may form intra-cellularly thereby damaging the cell membrane integrity and increasing the leaching of cell solutes from the seeds (Fenner, 1985; Copeland & McDonald, 1995). Under these circumstances all the species, with the exception of *S. microphylla,* showed evidence of structural membrane damage. This evidence included the rapid imbibition of seeds, the exudation of coloured leachates and the extensive fracturing of the seed surfaces. Alterations in seed membranes presumably increased the accessibility of the seed embryo-endosperm substrates to microorganisms and may compensate for any reduction in microbial activity caused by intermittent freezing events.

The effect of freeze-and-thaw cycles on the nitrogen dynamics showed no consistent pattern in the surveyed species. Schimel and Clein (1996) also reported a similar disparity in nitrogen release from four soils exposed to cyclical freezing events. The difference was attributed to the initial nitrogen content of the soil organic matter. The quantity of nitrogen release was positively associated with the initial soil nitrogen availability. No evidence of this trend was found in the present study. This may be a consequence of the different medium studied. The organic matter within the soil is presumably at a more advanced stage of decomposition limiting the applicability of soil experiments to fresh litter experiments.

The net nitrogen mineralisation from the seeds of *Nothofagus* and leguminous species subjected to repetitive freeze-and-thaw cycles was comparable or higher than those maintained at a constant temperature. The repetitive perturbations of freeze-and-thaw events probably maintained a reduced level of microbial activity and growth. This climatic control restricts the increment of microbial biomass and thus the accumulation of mineral nitrogen into microbial tissue. Further, the microcosms were analysed within 12 hours of the last freezing event. This limited the period available for the microbes to respond to more favourable conditions and therefore immobilise nitrogen.

A similar increase in net nitrogen mineralisation has been noted in soil studies. De Luca *et al* (1992) measured a three-fold increase in the mineral nitrogen leached from the soil after exposure to a single freeze event (-20°C for 7 days). The increased nitrogen mineralisation was prescribed to the release of nutrients from the disrupted microbial biomass. The relative increase in nitrogen as measured by De Luca *et al* (1992) greatly exceeded that measured in the present study . This may be due to the extreme temperatures and the longer duration of the freeze event used by De Luca *et al* (1992).

The periodic drying of all test species caused a reduction in the mass loss and nitrogen release. Clein and Schimel (1994) reported a comparable reduction in decomposition. Intermittent drying events significantly reduced the carbon mineralised from birch leaf litter over two months. The effect of wet-and-dry cycles on seed decomposition varied between species. The mass loss relative to the control (constant moisture) from *P. contorta* seeds was reduced by 22% whereas with *C. scoparius* it was only reduced by 4%. This is consistent with the work of Taylor and Parkinson (1988) on *Pinus* and aspen leaf litter. Exposure to wet-and-dry cycles for one month accelerated aspen litter decomposition, whereas no change was measured in the *Pinus* species. They postulated this difference to be a consequence of the divergent physical properties of the leaves. Aspen litter was considered more susceptible to leaf fracture following repetitive wet-and-dry cycles thus increasing the rate of decomposition.

The simulated wet-and-dry cycles in the present study were expected to arrest microbial activity during desiccation periods, as moisture is essential for microbial metabolism. Evidence for this was exhibited in the seeds of *N. solandri*, *N. fusca*, *C. scoparius*, *P.radiata*

and *P. contorta*. The mass loss incurred at 180 days under cyclical moisture was similar to that at 90 days under constant moisture (Table 4.4). These results infer either no effect on the persisting microbial populations' efficiencies to degrade seed substrates or alternatively any adverse effect on microbial activity is negated by the positive effect on substrate accessibility. The dehydration events simulated in the present study were likely to cause seed coat rupture through the repetitive expansion and contraction of seed coat cells (Fenner, 1985) and result in the production of reactive forms of oxygen that may damage seed membranes, proteins and nucleic acids (Copeland & McDonald, 1995).

The balance between the cost incurred due to reduced microbial activity during drying events and the benefit from the increased structural damage to the seed outer layers determines if wet-and-dry cycles accelerate or impede the rate of seed decomposition. The benefit obtained by wet-and-dry events is a function of the seed coats effectiveness as a barrier to the microorganism under constant conditions in the absence of other scarifying processes. Wet-and-dry cycles tend to facilitate the decomposition of species with a high mass allocation to seed coats, such as *C. scoparius* and *U. europaeus*. The benefit gained from increased accessibility to the seed's embryo-endosperm fraction outweighs the cost to the decomposition process of reduced microbial activity during intermittent dry periods. The inverse occurs in species with a smaller mass allocation to the seed coat.

Previous studies have reported a successive reduction in microbial diversity and activity in soils following exposure to repetitive hydration and desiccation events (Orchard & Cook, 1983; Van Gestel *et al*, 1993). The lethality is related to the physiological stresses caused by rapid increases and decreases in microbial cell water potential (Kieft *et al*, 1987). The drying effect is likely to favour fungal mycelia (Schimel *et al*, 1999) and bacterial cells in the stationary phase of growth (Chen & Alexander, 1973). In the present study, this drying effect on microorganisms may not be as severe due to the moderate temperatures employed. Previous soil experiments often used higher temperatures (40-80°C) to facilitate rapid drying (Jager & Bruins, 1975; Bottner 1985), whereas the microcosms in the present study were exposed to drying winds at only 20°C. This gradual drying may be sufficient to allow the microbial communities to acclimatise to the ensuing desiccation period. Chen and Alexander (1973) reported that the exposure of microbes to low osmotic conditions prior to desiccation significantly reduced the lethality of drying.

The closed conditions of the microcosms exposed to episodic stresses have the potential to alter the decomposition rate via its selective effect on microorganisms. In the natural environment the reduction in the microbial diversity is less pronounced due to the potential for re-inoculation from the lower soil horizons. Conversely, no re-inoculation was simulated in the microcosms exposed to wet-and-dry and freeze-and-thaw events. The residual microbial populations may be devoid of specialised guilds thereby reducing their efficiency in degrading substrates. The successive reduction of microbial diversity was proposed by Clein and Schimel (1994) to account for the reduced long term decomposition of birch leaf litter following desiccation. The loss of specialised enzymatic systems, that are likely to aid in the mobilisation of the more refractory forms of organic compounds, may be increasingly more important in the latter stages of decay.

4.3.3 Organisms

The results of both the endophyte and the native soil inoculum microcosm experiments must be interpreted with caution because the results may be potentially confounded by the non-simultaneous nature of the experimental design. The decomposition variables measured were compared with the results obtained from seeds decomposed with Ilam soil inoculum five months earlier. Therefore, the comparative increases or decreases may not be indicative of differential degradation efficiencies of microbial populations but rather be reflective of the different physiological status of the seeds.

The seed harbours, on or within its seed coat, an array of microbiota. van Leeuwen (1981) speculated that the seed endophytes of *Cirsium vulgare* were adapted to utilise the seed substrate. Accordingly, the seed-borne microbial populations would be expected to display a superior efficiency in exploiting the seed substrates. The limited work on endophytes in the present study found that the rate of nutrient release was generally comparable with microcosms containing seed and soil microorganisms. The comparable ability of endophytes and soil microbes to decompose litter substrates was corroborated by Walker (1994), who found no difference in the decomposition of floral litter in either the presence or absence of soil microorganisms. In the absence of soil microorganisms the seeds of *P. radiata* released 10% more nitrogen after 180 days incubation than seeds in the presence of soil

microorganisms. However, insufficient experimental work was done to speculate on the cause for this increased nitrogen release.

Soils are composed of a diverse array of microbial enzymes. This diversity is thought to reflect both the mosaic of primary and secondary resources entering the soil and the microbes nutritional versatility and their rapid response to changes in the environment (Heal & Dighton, 1985). A number of litter studies have observed significant location effects on the rate of leaf litter decomposition (Lamb & Florence, 1975; Howard & Howard, 1980; Smith *et al*, 1998; Vesterdal, 1999). The discrepancy between sites has been speculated to involve the varied diversity and efficiencies of the microbial communities. Further, taxonomic studies have found large compositional differences in the microbial communities between various soils (Witkamp & van der Drift, 1961; Latter & Heal, 1971). Conclusive evidence of divergent microbial diversity between soils is limited by a number of factors. These include the inadequacy and selectivity of isolation techniques, the controversial application of the species concept to bacteria and the difficulties in separating the potentially confounding effects of the abiotic environment on the microbial communities.

The divergent ability and efficiency of individual soil microbes to exploit organic substrates has been well established. An example of this was illustrated in the work of Colpaert and Van Tichelen (1996). Their research contrasted the degradative ability of ectomychorizal and litter-decomposing Basidiomycetes fungi on *Fagus sylvatica* leaf litter. The Basidiomycetes species displayed increased penetrative growth form and enzymatic efficiency to degrade *F. sylvatica* leaf litter. This is further illustrated by the varying efficiencies of soil microbial populations to degrade seeds coats (Gogue & Emino, 1979; Kirkpatrick & Bazazz, 1979). Gogue and Emino (1979) surveyed 3 soil microbial species for their efficiency to degrade the seed coat of *Albizia julibrissin*. The effectiveness of microrganisms species in altering the seed coat increased from *Phythium* to *Fusarium* to *Rhizotonia*.

Salonius (1981) reported that the full soil microbial populations demonstrated increased degradative efficiencies compared to microbial populations of reduced diversity. The presence or absence of specific microbial enzymatic systems has the potential to alter the rate of decomposition (Moucawi *et al*, 1981; Wardle & Lavelle, 1997). The current study postulates two divergent views regarding the soils' enzymatic potential. First, all soil systems

contain congruent enzymatic diversity. However, the expression of enzymatic systems is constrained by the physico-chemical environment consequently altering the soils degradation capacity and efficiency. Second, the soils contain inherently different enzymatic potential. Even under conditions conducive to enzymatic expression the absence of specific guilds of enzymes will impede the rate of decomposition. The microcosm experiments performed in this study made the assumption that all soil microbial communities contain the same assemblage of enzymes with similar efficiencies to degrade the seed substrates. Accordingly, the measured decomposition rate may be an artefact of the microorganisms present in the Ilam soil inoculum. This was tested by comparing the decomposition rate of *C. scoparius*, *N. solandri* and *P. radiata* whole seeds inoculated with the standard Ilam soil the inoculum and the native soil inoculums.

The seeds incubated with the native and Ilam soil inoculums incurred similar mass losses. This infers Ilam soil inoculum contain sufficient enzymatic capacity and efficiency to degrade the seed substrates. These finding do not support the hypothesis of van Leeuwen (1981) who predicted that the soil beneath a parent plant would be superior at decomposing seed substrate thus increasing seed mortality. This hypothesis was based on the premise that the accretion of specific plant substrates into the soil system would select for microbial population competitively superior at exploiting these substrates.

The lack of a soil inoculum effect may be a result of the high quality substrate the seed litter represents. The seeds of the species in the present study are predominantly composed of embryo-endosperm tissue, which represented 53-92% of the seed mass. The metabolism of this energy- and nutrient-rich fraction requires cosmopolitan r-selected microbes. Most soils would be expected to show similar efficiency in degradation of the high substrate quality component of the seeds. As decomposition progresses the importance of specialised enzymatic systems present in the native soils may increase with the accumulation of recalcitrant materials (Heal & Dighton, 1985; Bianchi & Bianchi, 1995). It was hypothesised earlier that specialised microbes might also be important in the early stages of seed decomposition due to the physical barrier imparted by the recalcitrant chemistry of the seed coat. The comparable influence of soil inoculums can be interpreted as 1) contrary to this hypothesis; 2) the soil inoculums contained equivalent enzymatic capacity and efficiency to degrade the seed coat substrates; 3) specialised enzymatic systems may be present in the seed

coat and therefore dispersed with the seed; 4) the role of specialised systems in the degradation of the seed coat may be species- and condition-specific. The specialised enzymatic systems required to breach the seed coat are expected to be important in the absence of other scarifying processes. The microcosms were incubated under a fluctuating temperature regime thereby potentially scarifying the seeds. The presence of this scarifying mechanism may be masking any promotory effect of specialised soil microbial populations.

A significant difference was found in the rate of nitrogen release between soil inoculums. The quantity and rate of nitrogen release was greatest in the presence of native soil inoculum. This implies that the release of seed nitrogen and hence its availability to plants may be greater in the site where the species is dominant. The use of the Ilam soil inoculum in the present study made the assumption that all soils contain a similar enzymatic potential. The assumption appears valid for the turnover rates in the first six months of decomposition. However, the inoculation technique may have lead to an underestimation in the rate of nitrogen release. It would be of value to repeat such inoculum experiments over longer time periods.

4.4 Seed decomposition in the natural environment

The seeds incubated *in situ* estimated the collective action of leaching and microorganisms on seeds exposed to conditions prevailing in the natural environment. The rate of mass loss varied substantially between species. The decay constants ranged from 0.01 in *S. microphylla* to 2.69 in *U. europaeus*, reflecting the divergent chemical and physical properties of the seeds. Nelms and Twedt (1996) reported similar variation in the rate of seed degradation incubated *in situ*. They surveyed the mass loss from 3 crop and 8 weed seeds incubated in flooded agricultural fields for 4 months. The calculated rate of decay ranged between 0.31 in Red rice and 2.27 in corn seeds. The rate of seed decomposition is within the upper limits of leaf litter decay rates as shown in Table 4.5. The seeds of *S. microphylla* and *C. scoparius* incurred only minimal mass losses that cannot be separated from the reductive effects of leaching. The negligible mass losses measured are likely to be a consequence of the hard seed coat that has been discussed earlier.

Table 4.5 The decay rate (k) of leaf litter over 12 months.

Reference	Species	Decay rate (k) (0-365)
Berg & Staaf (1981)	*Salix* species *Pinus silvestris*	1.83 0.12
Smith *et al* (1998)	*Leguminosae* species *Pinus caribea*	1.13 0.39
Moro & Domingo (2000)	*Adenocarpus decorticans* *Pinus pinaster*	0.83 0.13

The rapid decay rate of seeds is evident in the *Pinus* species, *Nothofagus* species and *U. europaeus* with a 95% turnover estimated to occur within 2.5 years. The mass loss from these seeds can be prescribed to both leaching and microbial catabolism because the values exceed the mass of the seed's labile fraction. These estimates are net measurements of the seed population and make no inferences about the individual seed's rate of turnover. This is illustrated in the seeds of *U. europaeus*. This species is an invasive weed in New Zealand with a persistent soil seed bank (Egunjobi, 1971; Wilson, 1990). The seeds of *U. europaeus* are considered highly persistent in the subterranean environment with viability being retained for over 30 years (Zabkiewicz, 1976; Baskin & Baskin, 1998). The extensive mass loss measured in the present study appears contrary to these estimates. This inconsistency reflects

the variability within a seed population to longevity reducing factors. Individual seeds within the bags retained their lustre and showed no indication of microbial colonisation. The residual seeds in the litter bags were likely to have experienced only minimal mass loss. However, the proportion of the population being degraded masked their lack of decomposition. It is possible that these remaining seeds, in their entire state, will persist in the soil for decades thereby not refuting previous reports relating to *U. europaeus* seed longevity.

The large variation between litter bag replicates of *Pinus* species after 90 days were due to insect larvae attack. These attacks were localised with respect to the litter bag replicate and the soil depth. The source of the insect larvae is likely to be of soil origin as no larvae were observed in the microcosm experiments. Alternatively, the larvae may have originated from the seed however conditions appropriate for their development were not present. It is likely that the presence of larvae accelerated the rate of seed decomposition. The macerating activity of larvae extensively fragmented the seed material. The increased exposure of the seeds internal surfaces facilitates the access of water and microbial enzymes to the embryo-endospermic tissue. As demonstrated in the microcosm experiments, insect-microbe interactions with seeds are important in promoting the rapid turnover of seeds in the natural environment.

The measured decomposition rate *in situ* was comparable to that measured in microcosms exposed to diurnal temperature fluctuations (5-15°C). This is inconsistent with previous *in situ* leaf litter experiments which generally reported a comparative reduction in the decomposition rate (Day, 1983). This was illustrated in the complimenting studies of Lousier and Parkinson (1976) and Taylor and Parkinson (1988). The rate of aspen leaf litter decomposition in microcosms was 6 times greater than that for leaf litters *in situ* incubated for the equivalent period. The comparable decay rates of seeds decomposed *in situ* and microcosm experiments can be attributed to the additional decomposition processes acting synergistically with microbes in the soil environment. These include leaching of water-soluble material, the actions of insect larvae and the altered structural integrity of the seed coat resulting from the action of scarifying mechanisms in the soil environment. Presumably these effects counteracted any detrimental influences that the dynamic subterranean environment had on the degrading activity of the microorganisms.

Most species showed a net release of nitrogen over time, with *S. microphylla* being the exception. The rate of nitrogen release was generally linear with seed mass loss. These results support the prediction of nitrogen dynamics proposed by Berg & Staaf (1981). Nitrogen release is hypothesised to occur from substrates exhibiting C:N ratios between 20 and 30. An initial leaching phase of rapid nitrogen loss on accession into the soil environment was not evident in the surveyed seeds. This contrasts with the results from numerous leaf litter experiments (Gosz *et al*, 1973; Day, 1983; Polglase *et al*, 1992; Prescott *et al*, 1993). The lack of a distinct leaching phase is likely to be a result of the impermeable nature of the seed coat and its dominant role in governing the pattern of decomposition. As indicated in the water-soluble experiments, leaching from whole seeds is only minimal. It is likely that leaching is a gradual process in seeds corresponding with the progressive degradation of the seeds protective outer layers via biotic and abiotic scarifying mechanisms. The alteration in the seed coat and thus the release of its labile component is likely to follow a sigmoidal pattern. This is evident in the data. It however, cannot be separated from the actions of microbes as the increasing alteration of the seed testa facilitates both microbial exploitation and water accessibility to the embryo-endospermic tissue.

The most extensive loss of nitrogen was measured in the seed of *P. radiata*. Following 180 days *in situ* the only residual material remaining in the litter bags was the seed coat. The mass (71%) and nitrogen (94%) loss equated to the mass and nitrogen contained within the embryo-endosperm fraction, thus supporting this observation. A comparable rapid turnover rate of seeds and the observed predominance of the seed coat fraction in residual material was reported by Zackrisson *et al* (1999). Pre-germinated heat-treated seeds of *Picea abies* incubated *in situ* for 90 days resulted in nearly 60% mass loss and 65-75% release of the seed nitrogen. These values exceeded the decomposition variables for all species measured, after 90 days incubation, in the present study. This is probably a reflection of the preconditioning treatment prior to their placement *in situ*. The *P. abies* seeds were germinated and heat-killed. This would have reduced the effectiveness of the seed coat as a barrier to microbial exploitation, thereby artificially accelerating the rate and pattern of seed decomposition.

The seeds of *S. microphylla* displayed net nitrogen accumulation after 90 days *in situ*. The nitrogen content increased by 18%. Such an accumulation is predicted to occur at a low

nitrogen concentration. This is at variance with the initial 3.01% concentration of nitrogen in *S. microphylla* seeds. However, the measurement of the total seed nitrogen is not indicative of the nitrogen presently available to microorganisms (Staaf & Berg, 1982) due to the impermeability and impenetrability of the seed coat. The low quality outer surfaces of the seed are effectively the substrates being decomposed. The 0.9% nitrogen concentration of the seed coat renders the substrate within the 0.3-1.4% limits for nitrogen accumulation reported by Berg and Staaf (1981). The increase in nitrogen represents approximately 2 mg of additional nitrogen. This may be a result of microbial tissue (Heal *et al*, 1982). Microbial tissue was estimated by Clinton *et al* (1999) to contain between 1-6% nitrogen. If the increase was purely attributable to microbial inputs, at least 33 mg of microbial biomass is required. The seeds showed no evidence of microbial colonisation therefore it is likely that a combination of nitrogen accumulation mechanisms is responsible for the increased nitrogen content. These may include nitrogen fixation, throughfall, precipitation (Grier, 1978) and absorption of leachates (Heal *et al*, 1982).

The depth of burial on mass loss was only significant in the seeds of *N. solandri*. The decay rate at the surface and 10 cm in the soil profile was 1.29 and 1.61 respectively. A similar trend of increased mass loss with depth was found in *P. radiata*, *N. fusca*, *P. contorta* and *C. scoparius*. However, the effect was only slight or the variability between replicates too great to detect a significant effect of depth. Witkamp and Olson (1963) reported a similar increase in decomposition with depth. The decomposition rate of non-confined *Q. alba* leaf litter within the litter layer exhibited a 27% greater mass loss than the corresponding leaves incubated on the soil surface. The increased decomposition with depth reflects the conducive environment for microbial activity (Witkamp, 1966; Anderson, 1973; Taylor & Parkinson, 1988). The surface of litter layer is the interface between the soil and the atmosphere. Therefore it experiences greater fluctuations in soil moisture and temperature (Taylor & Parkinson, 1988). As a consequence the microbial activity at the surface is reduced due to moisture fluctuations which negatively effect their decomposition efficiency (Swift *et al*, 1979).

The minimal effect of soil depth recorded in the majority of the test species may be a result of the micro-environmental effects of the litter bags. Studies monitoring the conditions within the litterbags have reported the artificial retention of water (Witkamp & Olson, 1963; Lousier

& Parkinson, 1976). The increased constancy of moisture within the litterbag may reduce the desiccation effects on the microbial community thereby accelerating the rate of decomposition. This artefact of the litter bag technique restricts its simulation of the natural conditions on the soil surface. Additionally, the reduced effect of soil depth may be a reflection of the seeds intrinsic substrate properties. The beneficial effects of the modified physical barriers of the seed coat may counteract the detrimental effects of the varying abiotic conditions at the soil surface. The balancing of these costs and benefits has been discussed earlier with regard to wet-and-dry and freeze-and-thaw cycles simulated in the microcosm experiments.

The litter bag technique is limited by its lack of resolution of the intrinsic and extrinsic factors governing the rate and pattern of decomposition due to the complexity of the natural environment. The technique does not allow complete simulation of the conditions found in the natural environment. The litter bag micro-environmental effects (Witkamp & Olson, 1963), the reduced contact with the soil medium (Witkamp, 1966), the restricted movement of certain size-classes of soil organisms (Cornelissen *et al*, 1999), the concentration of seed substrates and the artificial retention of seeds on the soil surface (Lousier & Parkinson, 1976) are uncharacteristic of the decomposition conditions present in the natural environment (Heal *et al*, 1997). Consequently, recognition of the above limitations is essential prior to any extrapolation and inferences of microbial seed decomposition in ecosystem functioning

The efficiency of seed degradation varied between soils. The mean decay rate for all species increased from Hawdon (1.08) to Darfield (1.65) to Lincoln (1.74) corresponding to a 95% turnover within 2.8, 1.8 and 1.7 years respectively. The moisture and temperature conditions used were standardised for all three soils. Therefore, the varied rates of decomposition are attributed to the effects of the soils' chemical and physical properties on the degradative activity of the microbial community. Numerous litter decomposition experiments have reported a significant site effect on decomposition rates (Witkamp & Olson, 1963; Lamb & Florence, 1975; Howard & Howard, 1980; Smith *et al*, 1998; Vesterdal, 1999). The cause of these disparities is speculated to involve biological differences. However, studies performed in the field are unable to separate local climatic influences on the microbial efficiency at degrading plant residues. Laboratory studies in which the soil has been maintained at a homogenous temperature and moisture content have also observed varied rates of decay

between soils. Jones (1999) measured the degradation efficiency of amino acid in 10 different soil types. The half life of amino acids in the soil ranged between 0.4 and 2.6 hours, illustrating the divergent efficiency of soils in degrading organic compounds.

The reduced degradation efficiency of the Hawdon soil may be related to the monoculture of the prevailing vegetation and the soil pH. It is feasible that microbial diversity is reduced by the monoculture of *N. solandri*. The heterogeneity of substrates entering the soil system is postulated to be a driving variable of soil microbial diversity. The selective effect of the relatively homogeneous nature of the substrates entering the detrital system at the Hawdon site may have resulted in the soils being enzymatically incomplete. The low pH may have also negatively influenced the activity of the microbial community. Comparable reductions in the decomposition rate with increased acidity have been reported in previous research. Moucawi *et al* (1981) surveyed the degradation efficiency on glucose, cellulose and plant lipid extractives of three soils with varying pH. Decomposition was consistently reduced in the acidic soils. The retardation was speculated to involve the effect of pH reducing both the microbial diversity and activity. As a consequence, filamentous fungi and yeasts dominated the microbial community.

4.5 Nutrient flux through the seed litter

Large quantities of energy- and nutrient-rich seeds are periodically released into the soil. The results of this current study illustrate that microorganisms rapidly mineralise the organic forms of nitrogen in the seeds. As nitrogen is generally considered the most limiting nutrient to primary production in terrestrial ecosystems, this pool of nitrogen in the seed component of the plant litter may represent an important transfer of nitrogen between the above- and below-ground components of the ecosystem (Ovington, 1963; Nielsen, 1977; Pregitzer & Burton, 1991).

The potential flux of nitrogen through the seed fraction of the litter is discussed with respect to *N. solandri*. Litter fall estimates were compiled by Wardle (1984) for the Craigeburn Ranges, thus corresponding to the collection sites for *N. solandri* plant litter used in the present study. The leaf and twig litter estimates were from a single year litter fall survey

however no corresponding seed estimates were found. The seed litter input was estimated over an 18 year period, from the mean seed production during full mast years.

The yearly inconsistency between the litter fractions potentially reduces the value of such an estimate. The allocation of plant biomass to vegetative growth is likely to be reduced during full masting years. This trade-off between reproductive and vegetative tissue was illustrated in the litter of *Nothofagus truncata*. Alley *et al* (1998) reported that the total above-ground litter fall was relatively constant between years. However, the proportion of seed material in the litter varied between 0.5% to 14% of the total annual litter. This was compensated by changes in the leaf litter fraction.

The value of the litter nitrogen content and net nitrogen mineralisation from the present study were applied to the figures of Wardle (1984). The nitrogen release was estimated from pure ground litter under controlled conditions. It is likely that the rate of release is reduced in the natural environment due to the complex interactions between the diversity of substrates and the dynamic physico-chemical environment in the soil. Consequently, the decomposition rates from this study will not provide a precise estimate. The figures presented are therefore an approximation of nitrogen inputs and mobilisation from the leaf, twig and seed litter fractions.

The mass and nitrogen entering the soil system from the seed, leaf and twig litters is listed in Table 4.6. Despite only comprising 6% of the total litter mass, the seed fraction of the above-ground litters surveyed represents 25% of the total nitrogen. These values are within the range reported for the reproductive litters of the masting species *Acer saccharum*. Pregitzer and Burton (1991) estimated between 14-61% of the above-ground litter nitrogen was contained in the seed and flower components of *A. saccharum*.

Quantitatively, the leaf component of the *N. solandri* above-ground litter represents the largest input of nitrogen. However, the decomposition of the leaf in the present study indicates only a small proportion of nitrogen is released over 180 days. The low nitrogen release infers that the leaf nitrogen is in a recalcitrant form or the substrate quality of the leaf material results in a rapid immobilisation of nitrogen. The seed fraction appears to represent an important component of the litter with respect to nitrogen release. Seed nitrogen is in a

form that is readily mineralised and re-circulated into the soil system. The figures used for seed released into the soil were derived from full masting years. These years of large seed output are estimated to occur every 3-10 years (Wardle, 1984), therefore in non- or partial-mast years the contribution of seeds to litter nitrogen is likely to be reduced.

Despite the limitation of applying litter and decomposition data from different years, the estimated values for the seed fraction represents an important contribution to nitrogen inputs from above-ground litter. The seed component represents a major flux of nitrogen into the soil which is readily mobilised by the microbial community.

Table 4.6 *N. solandri* litter fractions mass, nitrogen and nitrogen release.

Litter component	Input kg $ha^{-1}yr^{-1}$	Nitrogen kg N $ha^{-1}yr^{-1}$	N release (180 days) kg N ha^{-1}
seed	253	7.4	3.7 (49.8%)
leaf	2650	14.3	0.5 (3.4%)
twig	1200	7.9	0.1 (0.9%)

Note:
- Figures in parentheses indicate the percentage of net nitrogen mineralisation measured in the microcosm studies.

The release of high quality seeds and the mobilisation of the associated nutrients in the soil may have indirect beneficial effects on plant fitness. The plant provisions seeds with a high concentration of energy and nutrients, but has minimal control over the fate of these reserves. The microbial decomposition of seeds appears to be a direct loss to the plant. However, the mobilisation of seed nutrients by soil microorganisms may enhance plant fitness by providing increasingly favourable conditions for seedling establishment. This fertilisation effect of decomposing seeds was demonstrated by Zackrisson *et al* (1999). The decomposed seeds of *Picea abies* resulted in an increased nutrient content and growth response of *Pinus sylvestris* seedlings. They postulated this fertilisation effects as a mechanism of the mast seeding strategy to create high fertility conditions which benefit surviving seedlings. This suggests a symbiotic relationship between the soil microbial decomposers and plant seed substrates. The relationship is not tight in the classical interpretation of a symbiosis. Instead the benefit is returned indirectly and over a longer timeframe to the plant offspring. This further illustrates

the intimate association and balance between above- and below-ground components of the ecosystem.

4.6 Overall conclusions

Energy- and nutrient-rich seeds are released periodically into the soil system. The chemical characteristics of the seeds indicated a high quality substrate for decomposer microorganisms. This was further ratified in the decomposition of seed substrates in both microcosm and *in situ* environments. The organic forms of nitrogen present in the seeds were readily mobilised by soil and seed microorganisms and are therefore potentially available for utilisation by other components of the ecosystem.

The thickness and integrity of the seed coat largely determined the rate and pattern of decomposition. Artificial and natural scarification mechanisms accelerated the rate of decomposition by breaching the protective outer surfaces, thereby increasing the accessibility of microbial enzymes to the high quality embryo-endosperm fraction.

The large periodic release of seeds coupled with the biochemical data acquired in this study emphasises the importance of the seed fraction with respect to the quantity and quality of nitrogen entering the soil system from above-ground plant litter. Exclusion of the seed component of litter in nutrient budgets may potentially lead to an underestimation of the transfer of energy and nitrogen between the above- and below-ground components of the ecosystem.

4.7 FUTURE RESEARCH

The findings of the present study provide a starting point for additional research into the microbial decomposition of seeds. Some areas for further research are outlined below:

- Complimentary estimates of the annual seed inputs into the soil with decomposition studies to quantify and qualify nutrient mobilisation from the seed fraction of litter.
- Integration of seed bank and decomposition studies to investigate the microbial fate of seeds.
- Extensive chemical analysis of the seed coat and the embryo-endosperm fractions to identify possible chemical characters that impart properties of seed resistance to microbial decomposition.
- Decompose the seed coat and embryo-endosperm fractions in isolation to investigate their suitabilities as a microbial substrate.
- Taxonomic and molecular studies to identify possible succession of specialist or generalist microbial associations exploiting seed substrates.
- Investigate the ecological implication of the pulse of seed nutrients into the soil system periodically by decomposing ^{14}C and/or ^{15}N labelled seeds. This would reveal the fate of the seed nutrients in the soil ecosystem.

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